Molecular Diagnostics

REAGENTS FOR OLIGONUCLEOTIDE LABELING

Rev. 4

ELITechGroup
A BRUKER COMPANY

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1. Introduction

Toolbox for oligonucleotide labeling

ELITechGroup® has been actively involved in the development of fluorescence-based nucleic acid probe technologies since the early 1990s. This experience has helped us better understand the challenges of nucleic acid probe manufacturing and the needs of end users. The proprietary reagents and techniques that we have been developing have two main goals in mind: first, to provide labeled probes with the desired physicochemical properties, and, second, to simplify the probes' synthesis and purification. To achieve these goals, we have put together a comprehensive toolbox for nucleic acid labeling which includes a wide range of unique reagents:

- **Fluorescent dyes** for signal detection
- **Quenchers** for signal control
- **Nucleic Acid Base Analogues (Super Bases)** for modulation of nucleic acid duplex stability
- **Universal Nucleic Acid Bases** to control hybridization specificity
- **Minor Groove Binders (MGBs)** to enhance DNA duplex stability and hybridization specificity
- **Intercalators** for modulation of nucleic acid duplex stability
- **Artificial Nucleic Acids** for orthogonal nucleic acid technologies
- **Lipophilic groups** for therapeutic nucleic acid applications
- **Spacers and Linkers** to assemble various nucleic acid probe components together and optimize their performance.

These are offered in a variety of reagent forms:

- **2-Cyanoethyl Phosphoramidites** for internal and 5'-end labeling
- **Synthesis solid supports** for 3'-end labeling
- **Activated esters** for post-synthesis conjugation with amine-containing substrates

In addition, all of our Super Bases are available as both 3'- and 5'-phosphoramidites to make them compatible with both standard (3'→5') and 'reverse' (5'→3') DNA synthesis approaches. The reverse approach offers additional synthesis flexibility, for instance, in situations when a synthesis solid support is only available for 5'-end labeling.

Toolbox applications: DNA probe-based detection chemistries

Numerous DNA detection technologies that rely on fluorophore-labeled nucleic acids have been developed in the last several decades. Simple end-labeled fluorescent probes are useful in heterogeneous assays wherein the target-probe duplex is separated from the excess probe before signal detection (e.g., nucleic acid microarray technologies). On the other hand, most of the nucleic acid amplification technologies (e.g., real-time PCR) are performed in a homogeneous format with no separation of components and require a fluorescence change during or after amplification for target detection. The table below contains examples of some DNA detection chemistries, with the emphasis on ELITechGroup's proprietary MGB technology. More detailed analysis of such chemistries can be found in References 1 and 2. Signal generation for all of these chemistries can generally be attributed to either hydrolysis or hybridization events. The hydrolysis type probes, when annealed to a target, are enzymatically degraded to separate the fluorophore ('Fluor') from a quenching ('Q') moiety, whereas hybridization probes/primers generate fluorescence signal via a hybridization-triggered conformational change. Hybridization probes and primers have the benefit of post-amplification melting curve analysis to confirm the specificity of amplification or detect target mutations.

Detection Chemistry	Type	5'	3'	Internal
MGB TaqMan	Hydrolysis probe	Fluor	Q-MGB	N/A
MGB Pleiades®	Hybridization probe	Fluor-MGB	Q	N/A
MGB Eclipse®	Hybridization probe	Q-MGB	Fluor	N/A
DSQ TaqMan	Hydrolysis probe	Fluor	DSQ	N/A
MGB FRET	Hybridization probe	Fluor-MGB	Q	Fluor
MGB Fluorogenic primer	Fluorogenic primer	Fluor-MGB	None	N/A
TaqMan*	Hydrolysis probe	Fluor	Q	N/A
Molecular beacon	Hybridization probe	Fluor or Q	Q or Fluor	N/A
Scorpion	Fluorogenic probe/primer	Fluor	None	Q and blocker
Endonuclease IV	Hydrolysis probe	Q	Fluor	N/A
LUX primer	Fluorogenic primer	None	None	Fluor
FRET probes	Hybridization probe	Fluor	Fluor	N/A

**TaqMan is a trademark of Roche Molecular Systems, Inc.*

Structurally, the degree of complexity varies among the chemistries. In most cases 5'- or 3'- fluorophore and quencher reagents are involved, as well as 5'- or 3'-MGB for MGB probes. In more complicated cases, internal dyes and spacers are required.

ELITechGroup offers a wide range of dye and MGB reagents for post-synthesis and on-line incorporation to meet the challenges of any of those chemistries. Most of our reagents are available as internal phosphoramidites for internal and multiple dye incorporation. We also offer amine and dye-modified nucleoside phosphoramidites that allow for internal modifications with minimal impact on duplex stability.

MGB TaqMan (Section 9, Figure 13A), **DSQ TaqMan** (Section 12), **TaqMan** probes, **Molecular Beacons,** or **FRET** probes can be synthesized using our quencher synthesis supports (Sections 3, 11, and 12), standard 3'-nucleoside phosphoramidites, and concluded with fluorophore phosphoramidites. Alternatively, **Endonuclease IV** probes, which require a 5'-quencher and a 3'-fluorophore with a special linker to increase mismatch discriminating ability (Ref. 3), are prepared starting from a fluorophore synthesis support followed by 3'-nucleoside phosphoramidites and a quencher phosphoramidite at the last step.

For probes with internal fluorophore or quencher (**Scorpions, LUX primers** or **MGB FRET** (Section 9, Figure 13D) we offer fluorophore/quencher internal phosphoramidites with a non-nucleoside linker or nucleoside phosphoramidites with fluorophore/quencher attached to a nucleobase.

To further increase flexibility, reduce the consumption of valuable MGB and fluorophore reagents, and improve product quality in some cases we recommend using 5'-nucleoside phosphoramidites (a.k.a. 'reverse' phosphoramidites) for probe synthesis. For example, **MGB Pleiades** probes (Section 9, Figure 13C) contain 5'-MGB, 5'-fluorophore, and 3'-quencher moieties. For this configuration, the most economical synthetic path is to start from a 5'-Fluorophore-MGB synthesis support (Section 13), follow with 5'-nucleoside phosphoramidites for a probe sequence and complete with a quencher phosphoramidite. Having the hydrophobic quencher incorporated at the last step of the synthesis provides an efficient handle for reverse phase HPLC purification. To accommodate this approach with the duplex enhancing **Super Bases** (Section 5) we offer 'reverse' 5'-phosphoramidites of the Super Bases as well. Similarly, **MGB Eclipse** probes (Section 9, Figure 13B) can be synthesized starting from an EDQ-MGB synthesis support (Section 11), followed by 5'-nucleoside phosphoramidites addition, and a fluorophore phosphoramidite at the last step. M**GB Fluorogenic primers** (Section 9, Figure 12), which are analogous to MGB Pleiades with an unmodified 3'-hydroxyl end, can also be synthesized using the 'reverse' phosphoramidite approach.

2. Fluorescent Dyes

ELITechGroup's dyes are available in several fluorescent colors ranging from blue to far red. Their excitation and fluorescence emission spectra (Figure 1 and Figure 2) have been optimized to match filter sets for most commercial fluorescence-based nucleic acid diagnostic instruments. With assay multiplexing in mind, the dyes have also been designed to maximize spectral separation and minimize channel cross-talk.

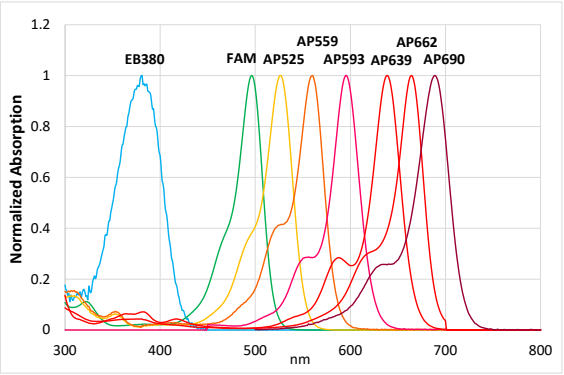


Figure 1 Absorption spectra of representative fluorophores.

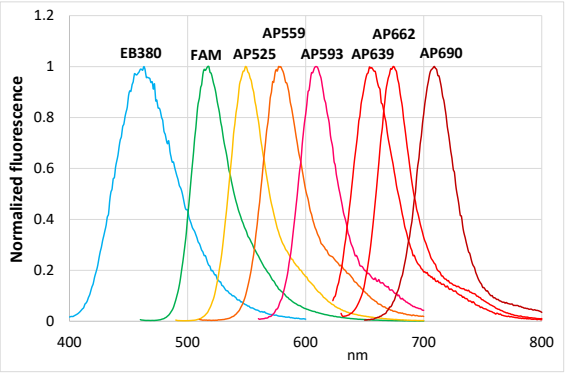


Figure 2 Emission spectra of representative fluorophores.

For most of our fluorophores, we offer three types of labeling reagents: 2-cyanoethyl dye phosphoramidites, dye-modified oligonucleotide synthesis supports for on-line dye incorporation and amine-reactive active esters for post-synthesis conjugation. These three types of reagents are detailed below.

2-Cyanoethyl Phosphoramidites

2-Cyanoethyl dye phosphoramidite chemistry is undeniably one of the most convenient methods for the preparation of labeled oligonucleotides. We offer dye phosphoramidites whenever they are synthetically accessible and sufficiently stable during oligonucleotide synthesis and deprotection. Our internal phosphoramidites contain a multifunctional hydroxyprolinol linker with one primary hydroxyl, which is blocked by the DMT group, one secondary hydroxyl for phosphoramidite incorporation, and an amine for dye attachment via an amide bond. This design allows dye incorporation at any desired position within a probe's sequence. In addition, if used in the last coupling cycle, the DMT group offers a convenient DMT-on purification handle, especially for dyes that do not significantly alter C18 HPLC retention.

Synthetically more accessible terminal phosphoramidites with a straight chain C6 linker are only suitable for single dye incorporation at the end of oligonucleotide synthesis but may be a viable alternative to the internal phosphoramidites depending on probe design and purification requirements.

Oligonucleotide Synthesis Supports

Dye-labeled controlled pore glass (CPG) and polystyrene (PS) supports are very economical methods for dye labeling. Depending on whether 5' or 3'-nucleoside phosphoramidites are used, this approach allows essentially quantitative 3' or 5' dye incorporation. All of our dye CPG or PS supports use the proprietary trifunctional hydroxyprolinol moiety, which provides a DMT-blocked primary hydroxyl (the starting point for oligonucleotide synthesis) and a secondary hydroxyl for connecting the dye to the synthesis support via a cleavable succinate linker.

Active esters

Most of our dyes are available as amine-reactive pentafluorophenyl (PFP) esters or fused lactones with activities similar to well-known NHS esters. Both non-aqueous and aqueous/organic conjugation conditions can be used.

Active esters: Post-synthesis conjugation using non-aqueous DMSO conditions

For conjugation applications, one particularly useful property of oligonucleotides is the solubility of their trialkylammonium forms in organic solvents such as DMSO. These forms can be prepared in a variety of ways. For example, a triethylammonium (TEA) form can be obtained by simple C18 reverse phase chromatography (which is usually done prior to a post-synthesis conjugation) using triethylammonium bicarbonate buffer. The volatile buffer can be readily removed by evaporation *in vacuo*. The resultant oligonucleotide (TEA form) is now soluble in DMSO and can be modified with a variety of agents under mostly anhydrous conditions. This approach allows use of conjugation reagents that are either insoluble or unstable in the presence of significant amounts of water, making the conjugation reaction more robust.

Another useful attribute of the non-aqueous protocol is that all oligonucleotide-related material can be easily precipitated by adding a solution of a sodium salt (such as iodide, perchlorate, etc.) in acetone, and separated from the bulk of unreacted dye, thus simplifying conjugate purification.

Overall, our experience indicates that when compared to aqueous/organic conditions, the non-aqueous conjugation procedure is more reproducible; it affords higher coupling yields and simplifies conjugate purification.

Active esters: Two-step conjugation procedures

Preparation of dye-labeled nucleic acid probes by post-synthesis conjugation may present significant challenges in terms of conjugation efficiency and separation of the resultant conjugate from unconjugated dye. The presence of unlabeled oligonucleotide will reduce the probe's quality and ultimately its performance. On the other hand, even minor contamination with unconjugated dye may lead to an undesired increase in background signal.

Conjugates can often be separated from the starting oligonucleotide and unconjugated dye by reverse phase HPLC. It is therefore desirable, for purification purposes, to use fairly hydrophobic dyes that provide significant changes in HPLC retention time. Excessive hydrophobicity, however, may lead to undesired dye aggregation and reduction in the probe's performance. To address this dilemma, we developed a two-step conjugation procedure. In the first step, the main conjugation reaction is

done using a transiently protected, hydrophobic form of a dye followed by simple reverse phase HPLC purification. In the second step, the conjugate is treated with a deblocking agent releasing the dye in a more hydrophilic form and purified again. Importantly, the dual HPLC purification procedure simplifies the removal of free, unincorporated dye. This approach is illustrated in Figure 3 for a conjugation reaction between the AquaPhluor 525 lactone (M830683) and an amine-modified oligonucleotide. The AquaPhluor 525 dye, in its fully deprotected form, is highly hydrophilic and provides no useful change in the retention time for HPLC purification. On the other hand, its intermediate has the phosphonate group blocked by the trifluoroacetamidobutyl (TFAAB) groups, which provide a very convenient purification handle. The blocking groups are removed at the next step by treatment with ammonium hydroxide generating the highly polar zwitterionic phosphonate.

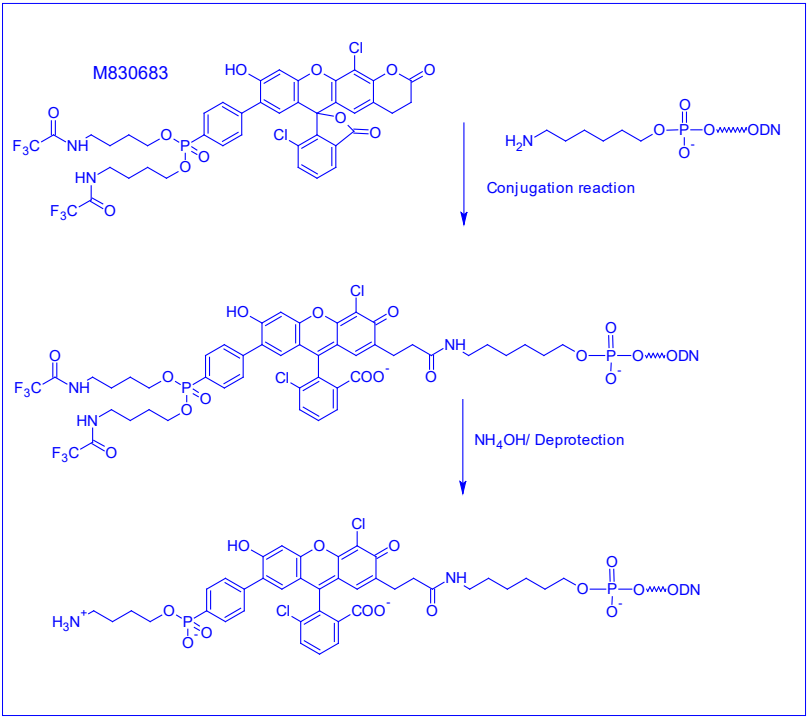


Figure 3 Two-step post-synthesis incorporation of AquaPhluor 525 using its active lactone form (AP525 lactone, M830683).

On-line incorporation of AquaPhluor® Dyes

Our proprietary AquaPhluor dyes have been developed for direct on-line incorporation of polar fluorescent dyes into oligonucleotide probes. In their form as labeling reagents, they possess latent phosphonate groups which are fully protected and neutral and, therefore, compatible with the phosphoramidite preparation and coupling chemistries. Upon completion of standard oligonucleotide synthesis, in their ultimate form the dyes are negatively charged and thus significantly more hydrophilic. The phosphonate chemistry of AquaPhluor dyes makes it possible to combine the versatility of automated oligonucleotide synthesis with the benefits of hydrophilicity. As an additional benefit, the phosphonate is a convenient attachment point for a variety of linking and functional groups. Based on how the phosphonate group is utilized, the AquaPhluor dyes can be divided into two classes. AquaPhluor 525, for example, has a phosphonate that is blocked with two TFAAB groups, one of which is eventually removed producing a highly hydrophilic zwitterion (Figure 4).

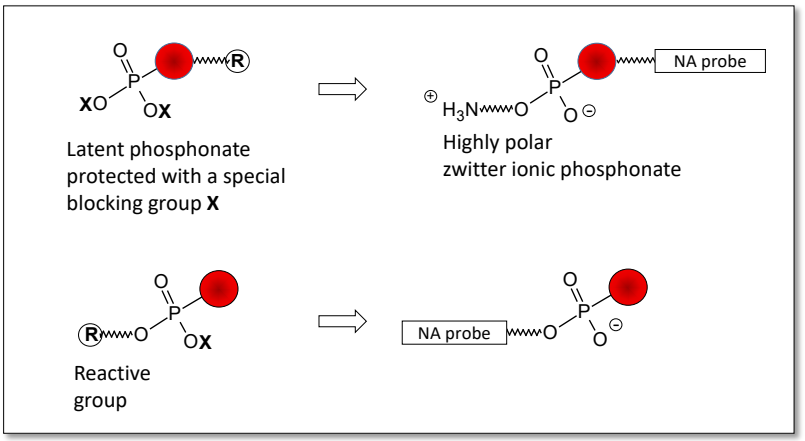
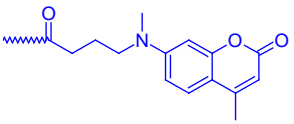


Figure 4 Two types of AquaPhluor chemistries for on-line dye incorporation.

In the second class, which includes AP559, AP593, AP570, AP639, AP642, AP662, AP680 and AP690 dyes, a reactive linking group is integrated as one of the phosphonate blocking groups. After incorporation of the dye into an oligonucleotide and treatment with deblocking agents, only the special blocking group is removed leaving the conjugation linkage intact. Both types of the dyes are compatible with phosphoramidite, active ester, and oligonucleotide chemistries.

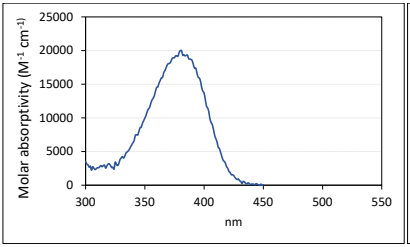
Epoch Blue 380

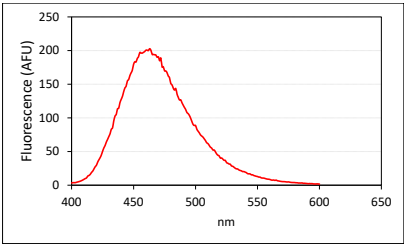
Acronym: EB380



Epoch Blue 380 is a UV light-excitable blue fluorescent aminocoumarin dye with excitation and emission properties similar to those of Marina Blue and Alexa Fluor 350. It is fully compatible with oligonucleotide synthesis and deprotection.

Absorbance maximum*	381 nm
Emission maximum*	460 nm
Extinction coefficient (260 nm)	7,000 $M^{-1}cm^{-1}$
Extinction coefficient (260 nm)	17,200 $M^{-1}cm^{-1}$ (Epoch Blue 380-HEG)
Extinction coefficient (381 nm)	20,000 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.24
Hydrophobicity by C18 chromatography***	22.0 (moderately hydrophobic)
pH dependence of dye fluorescence	Independent between pH of 5 and 8 (Appendix C)



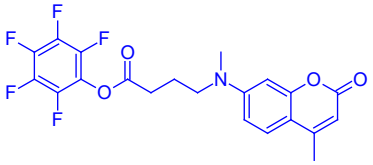


Available Epoch Blue 380 products	Product number
Epoch Blue 380 PFP ester	M830726
Epoch Blue 380 Internal Phosphoramidite	M830192
Epoch Blue 380-HEG Phosphoramidite	M830727
Epoch Blue 380 CPG	M830669
Epoch Blue 380 Polystyrene****	M830670 (bulk), M100441 (columns)

**Measured for T_8-Epoch Blue 380 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-Epoch Blue 380 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-EB380-HEG Dye Calibrator (M300585) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

Epoch Blue 380 PFP ester

Product number: M830726; CAS #: 2378004-31-8



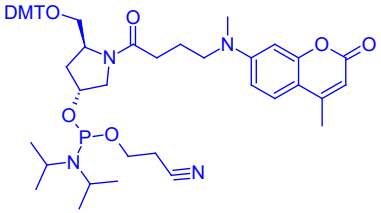
Formula	$C_{21}H_{16}F_5NO_4$
Molecular weight (reagent)	441.35 Da
Molecular weight (incorporated)	257.28 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830726	bulk
M830726-1MG	1 mg
M830726-10MG	10 mg

Epoch Blue 380 Internal Phosphoramidite

Product number: M830192; CAS #: 2378004-42-1

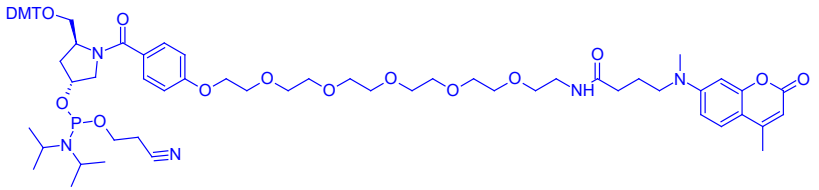


Formula	$C_{50}H_{61}N_4O_8P$
Molecular weight (reagent)	877.03 Da
Molecular weight (incorporated)	436.39 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 8 years
Available documents	Certificate of analysis (CoA)

Catalog number	Unit size
M830192	bulk
M830192-50UMOL	50 µmol
M830192-100UMOL	100 µmol
M830192-250MG	250 mg
M830192-1000MG	1 g

Epoch Blue 380-HEG Phosphoramidite

Product number: M830727; CAS #: 2378004-26-1

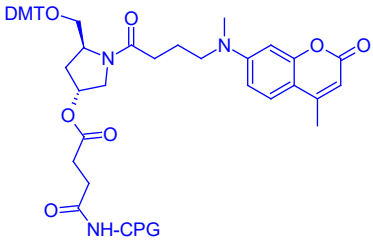


Formula	$C_{69}H_{90}N_5O_{15}P$
Molecular weight (reagent)	1260.45 Da
Molecular weight (incorporated)	819.83 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830727	bulk
M830727-50UMOL	50 µmol
M830727-100UMOL	100 µmol
M830727-250MG	250 mg
M830727-1000MG	1 g

Epoch Blue 380 CPG

Product number: M830669

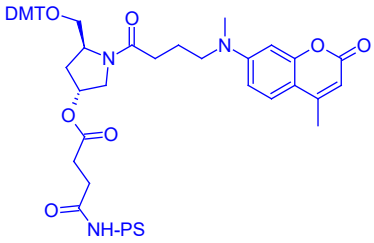


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	436.39 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830669	bulk
M830669-100MG	100 mg
M830669-250MG	250 mg
M830669-1000MG	1 g

Epoch Blue 380 Polystyrene

Product numbers: M830670 (bulk), M100441 (columns)



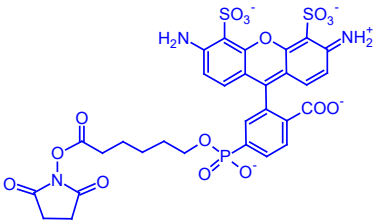
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	436.39 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830670	bulk
M100441-P3	Pack of 3 columns*
M100441-P10	Pack of 10 columns*
M100441-P30	Pack of 30 columns*
M100441-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AquaPhluor® 492 NHS ester

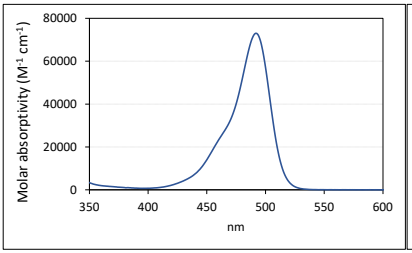
Product number: M830194; CAS #:2378004-15-8

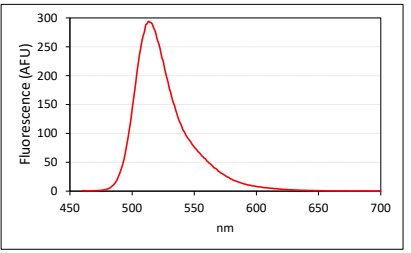


AquaPhluor 492 is a highly hydrophilic fluorescent dye with excitation and emission properties similar to Alexa Fluor 488 and fluorescein. Compared to fluorescein, AP492 is less pH-sensitive and more photostable.

AP492 NHS ester is suitable for conjugation reactions with amine-containing substrates.

Absorbance maximum	492 nm
Emission maximum*	513 nm
Extinction coefficient (260 nm)	13,500 $M^{-1}cm^{-1}$
Extinction coefficient (492 nm)	73,000 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	TBD
Hydrophobicity by C18 chromatography***	TBD (hydrophilic)
pH dependence of dye fluorescence	TBD



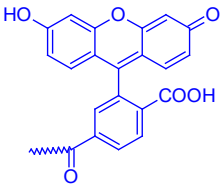


Formula	$C_{30}H_{25}Li_3N_3O_{16}PS_2$ (Li salt)
Molecular weight (reagent)	799.46 Da
Molecular weight (conjugated)	666.57 Da
Purity**** (reverse phase HPLC)	≥70%
Structure identity test	^{1}H NMR
Recommended coupling conditions:	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 3 years
Available documents	CoA, conjugation protocols

**Measured for T_8-AP492 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP492 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP492 from C18 column (Appendix A)*
*****Purity of this active derivative is verified by HPLC & NMR and usually exceeds 70%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

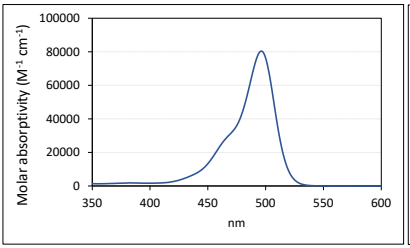
Catalog number	Unit size
M830194	bulk
M830194-1MG	1 mg
M830194-10MG	10 mg

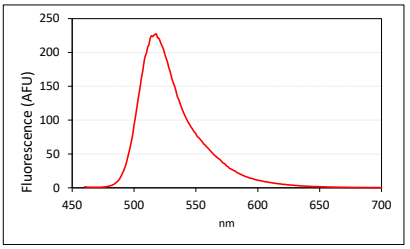
6-FAM



FAM (6-Carboxyfluorescein) is a popular green fluorescent dye compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum*	496 nm
Emission maximum*	517 nm
Extinction coefficient (260 nm)	33,300 $M^{-1}cm^{-1}$ (FAM-HEG)
Extinction coefficient (496 nm)	80,400 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.88
Hydrophobicity by C18 chromatography***	19.2 (hydrophilic)
pH dependence of dye fluorescence	pK_a 6.8-7.0 (see Appendix C)



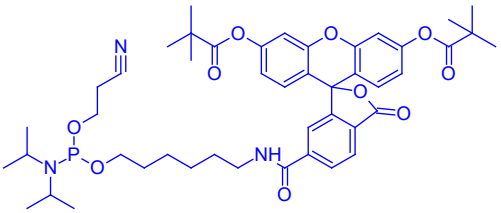


Available FAM products	Product number
6-FAM Phosphoramidite	M100011
FAM-HEG Phosphoramidite	M830100
FAM-HEG CPG	M830719
FAM-HEG Polystyrene****	M830718 (bulk), M100442 (columns)
FAM-dU 3'-Phosphoramidite	M830763
FAM-dU 5'-Phosphoramidite	M830764
FAM-Super I 3'-Phosphoramidite	M830783

**Measured for T_8-FAM-HEG conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-FAM-HEG conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-FAM-HEG Dye Calibrator (M300586) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

6-FAM Phosphoramidite

Product number: M100011; CAS #: 204697-37-0

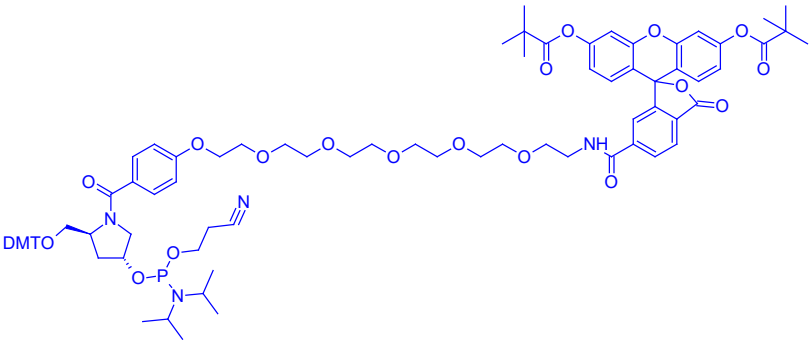


Formula	$C_{46}H_{58}N_3O_{10}P$
Molecular weight (reagent)	843.94 Da
Molecular weight (incorporated)	537.45 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M100011	bulk
M100011-50UMOL	50 µmol
M100011-100UMOL	100 µmol
M100011-250MG	250 mg
M100011-1000MG	1 g

FAM-HEG Phosphoramidite

Product number: M830100; CAS #: 2378004-27-2

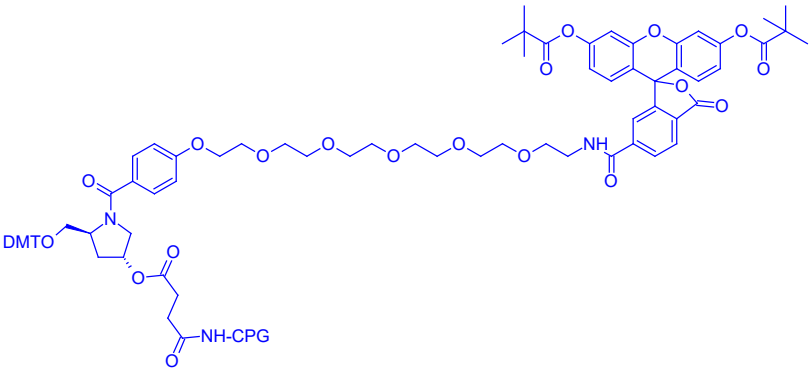


Formula	$C_{85}H_{101}N_4O_{20}P$
Molecular weight (reagent)	1529.70 Da
Molecular weight (incorporated)	920.84 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830100	bulk
M830100-50UMOL	50 µmol
M830100-100UMOL	100 µmol
M830100-250MG	250 mg
M830100-1000MG	1 g

FAM-HEG CPG

Product number: M830719

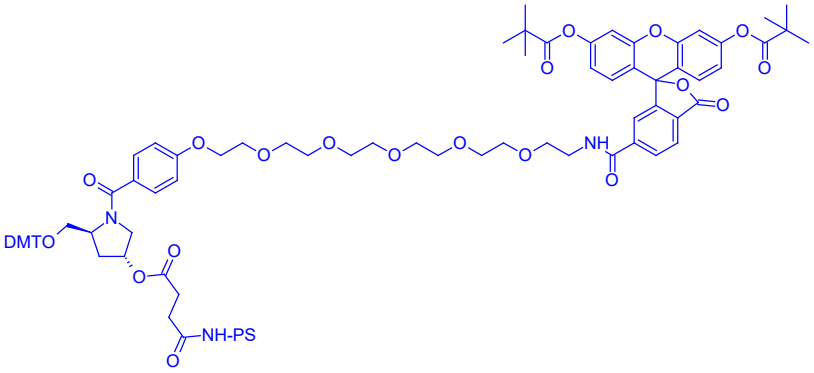


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	920.84 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830719	bulk
M830719-100MG	100 mg
M830719-250MG	250 mg
M830719-1000MG	1 g

FAM-HEG Polystyrene

Product numbers: M830718 (bulk), M100256 (columns)



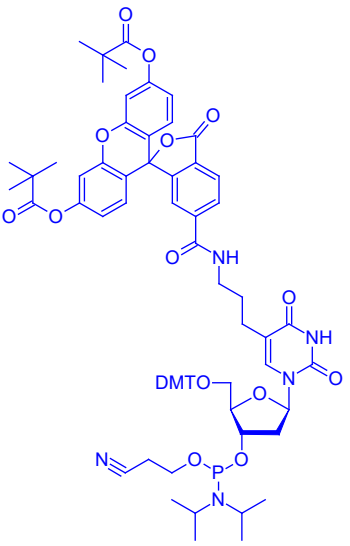
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	920.84 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830718	bulk
M100256-P3	Pack of 3 columns*
M100256-P10	Pack of 10 columns*
M100256-P30	Pack of 30 columns*
M100256-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

FAM-dU 3'-Phosphoramidite

Product numbers: M830763; CAS #: 2378004-02-3

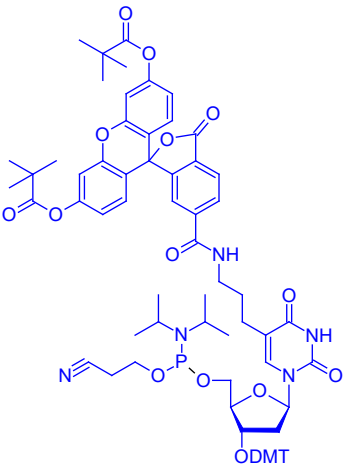


Formula	$C_{73}H_{80}N_5O_{16}P$
Molecular weight (reagent)	1314.41 Da
Molecular weight (incorporated)	705.56 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830763	bulk
M830763-50UMOL	50 µmol
M830763-100UMOL	100 µmol
M830763-250MG	250 mg
M830763-1000MG	1 g

FAM-dU 5'-Phosphoramidite

Product numbers: M830764; CAS #: 2378004-07-8

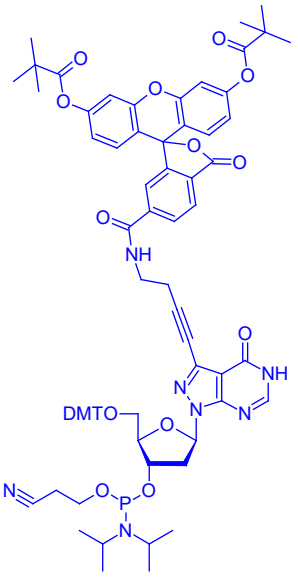


Formula	$C_{73}H_{80}N_5O_{16}P$
Molecular weight (reagent)	1314.41 Da
Molecular weight (incorporated)	705.56 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830764	bulk
M830764-50UMOL	50 µmol
M830764-100UMOL	100 µmol
M830764-250MG	250 mg
M830764-1000MG	1 g

FAM-Super I 3’-Phosphoramidite

Product numbers: M830783; CAS #: 2378004-06-7

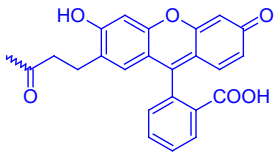


Formula	$C_{75}H_{78}N_7O_{15}P$
Molecular weight (reagent)	1348.43 Da
Molecular weight (incorporated)	739.58 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830783	bulk
M830783-50UMOL	50 µmol
M830783-100UMOL	100 µmol
M830783-250MG	250 mg
M830783-1000MG	1 g

Gig Harbor Green™

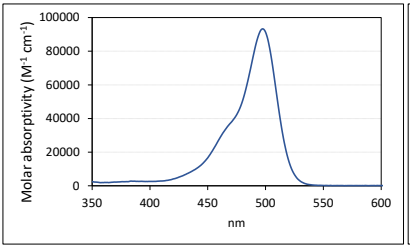
Acronym: GG

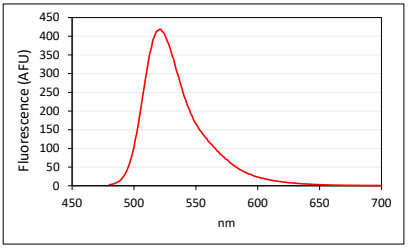


Gig Harbor Green is an alternative to FAM with similar excitation and emission properties.

Gig Harbor Green reagents are compatible with standard DNA synthesis and deprotection conditions.

Absorbance maximum*	497 nm
Emission maximum*	521 nm
Extinction coefficient (260 nm)	18,500 $M^{-1}cm^{-1}$
Extinction coefficient (500 nm)	93,200 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.85
Hydrophobicity by C18 chromatography***	21.5 (hydrophilic)
pH dependence of dye fluorescence	pK_a 7.4-7.5 (see Appendix C)



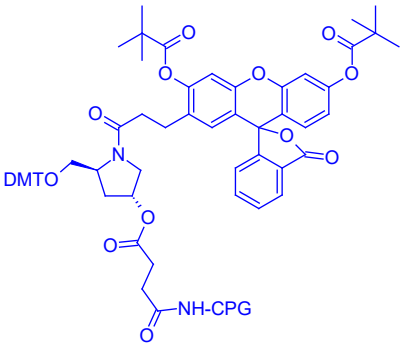


Available Gig Harbor Green products	Product number
Gig Harbor Green CPG	M830771
Gig Harbor Green Polystyrene****	M400090 (bulk), M100433 (columns)

**Measured for T_8-GG conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-GG conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-GG Dye calibrator from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

Gig Harbor Green CPG

Product number: M830771

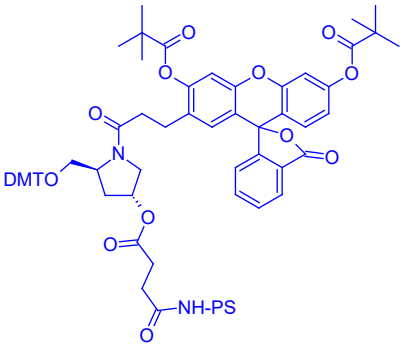


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	565.46 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830771	bulk
M830771-100MG	100 mg
M830771-250MG	250 mg
M830771-1000MG	1 g

Gig Harbor Green Polystyrene

Product numbers: M400090 (bulk), M100433 (columns)



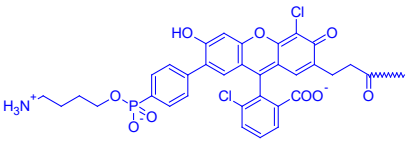
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	565.46 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M400090	bulk
M100433-P3	Pack of 3 columns*
M100433-P10	Pack of 10 columns*
M100433-P30	Pack of 30 columns*
M100433-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

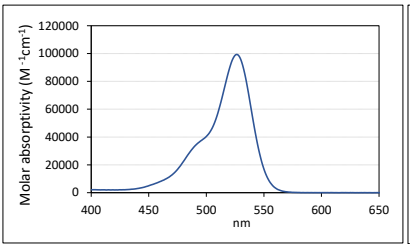
AquaPhluor 525

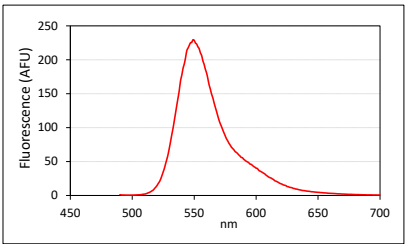
Acronym: AP525



AquaPhluor 525 is a hydrophilic fluorescent dye with excitation and emission properties similar to VIC, JOE, HEX and Alexa Fluor 532. It possesses a zwitterionic 4-aminobutyl- phosphonate group for increased hydrophilicity and reduced aggregation. The latent phosphonate is conveniently protected in the reactive forms of the dye.

Absorbance maximum*	527 nm
Emission maximum*	549 nm
Extinction coefficient (260 nm)	41,000 $M^{-1}cm^{-1}$
Extinction coefficient (527 nm)	99,300 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.73
Hydrophobicity by C18 chromatography***	18.6 (hydrophilic)
pH dependence of dye fluorescence	pK_a 6.8-7.0 (see Appendix C)





Available AquaPhluor 525 products	Product number
AquaPhluor 525 Lactone	M830683
AquaPhluor 525 Internal Phosphoramidite	M830199
AquaPhluor 525 Terminal Phosphoramidite	M830714
AquaPhluor 525-HEG Phosphoramidite	M100104
AquaPhluor 525 CPG	M830671
AquaPhluor 525 Polystyrene****	M830672 (bulk), M100276 (columns)

**Measured for T_8-AP525 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

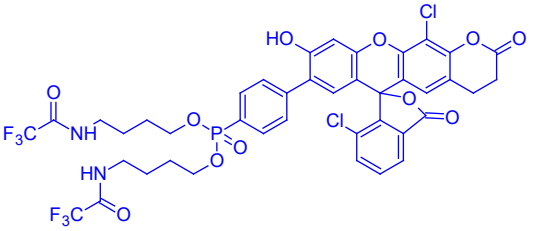
***Measured for T_8-AP525 conjugate (0.1 M Sodium tetraborate)*

****Percent acetonitrile required for elution of T_8-AP525 Dye Calibrator (M300587) from C18 column (see Appendix A for details)*

*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 525 Lactone

Product number: M830683; CAS #: 2378004-23-8



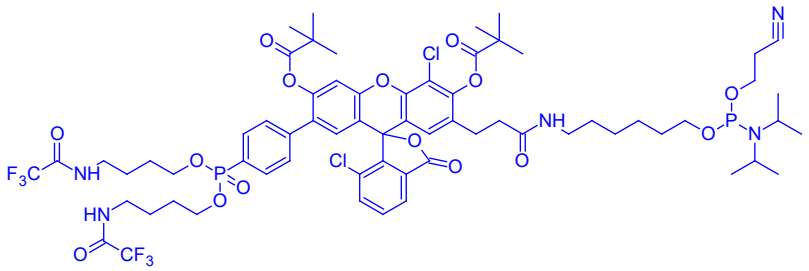
Formula	$C_{41}H_{33}Cl_2F_6N_2O_{11}P$
Molecular weight (reagent)	945.58 Da
Molecular weight (incorporated)	682.44 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Recommended deprotection conditions	See conjugation protocol
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830683	bulk
M830683-1MG	1 mg
M830683-10MG	10 mg

AquaPhluor 525 Terminal Phosphoramidite

Product number: M830714; CAS #: 876746-10-0

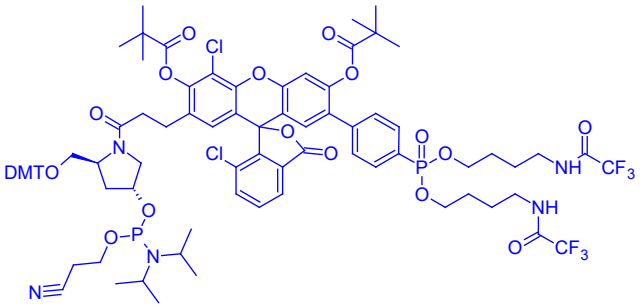


Formula	$C_{66}H_{81}Cl_2F_6N_5O_{15}P_2$
Molecular weight (reagent)	1431.23 Da
Molecular weight (incorporated)	861.59 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830714	bulk
M830714-50UMOL	50 µmol
M830714-100UMOL	100 µmol
M830714-250MG	250 mg
M830714-1000MG	1 g

AquaPhluor 525 Internal Phosphoramidite

Product number: M830199; CAS #: 2378004-28-3

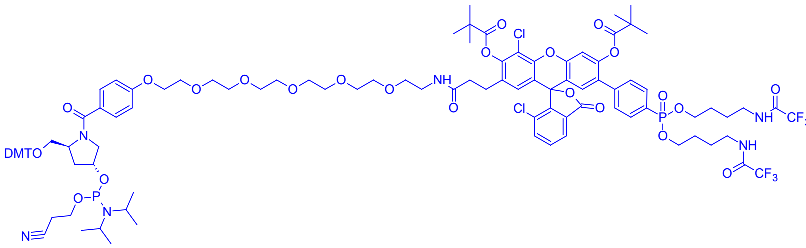


Formula	$C_{86}H_{95}Cl_2F_6N_5O_{18}P_2$
Molecular weight (reagent)	1733.54 Da
Molecular weight (incorporated)	861.55 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830199	bulk
M830199-50UMOL	50 µmol
M830199-100UMOL	100 µmol
M830199-250MG	250 mg
M830199-1000MG	1 g

AquaPhluor 525-HEG Phosphoramidite

Product number: M100104; CAS #: 2378004-19-2

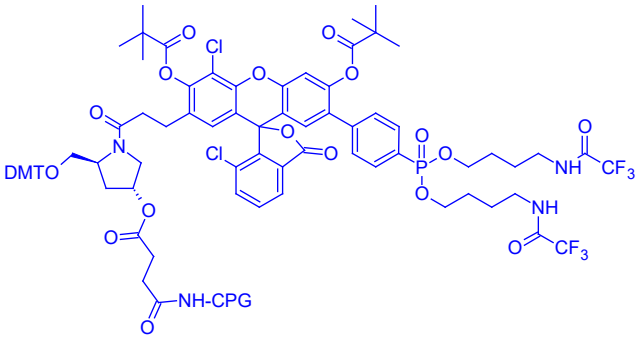


Formula	$C_{105}H_{124}Cl_2F_6N_6O_{25}P_2$
Molecular weight (reagent)	2116.98 Da
Molecular weight (incorporated)	1244.98 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	**Unit size**
M100104	bulk
M100104-50UMOL	50 µmol
M100104-100UMOL	100 µmol
M100104-250MG	250 mg
M100104-1000MG	1 g

AquaPhluor 525 CPG

Product number: M830671

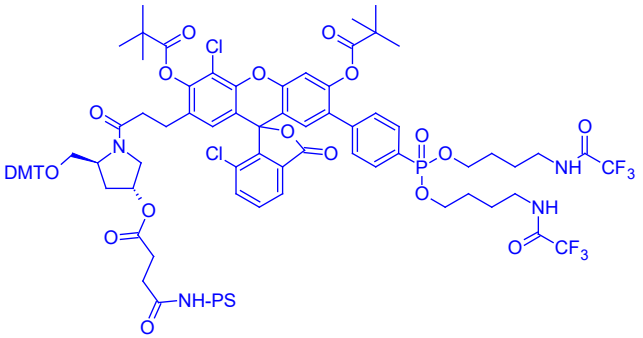


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	861.55 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830671	bulk
M830671-100MG	100 mg
M830671-250MG	250 mg
M830671-1000MG	1 g

AquaPhluor 525 Polystyrene

Product numbers: M830672 (bulk), M100276 (columns)



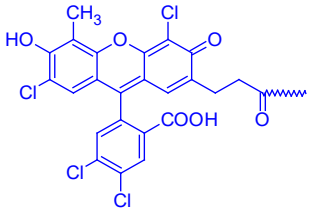
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	861.55 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 8 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830672	bulk
M100276-P3	Pack of 3 columns*
M100276-P10	Pack of 10 columns*
M100276-P30	Pack of 30 columns*
M100276-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

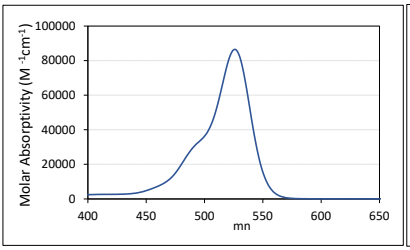
Yakima Yellow®

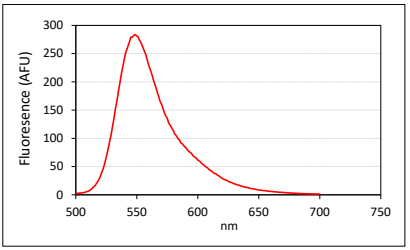
Acronym: YY



Yakima Yellow is a fluorescent dye with excitation and emission properties similar to VIC, JOE, HEX and Alexa Fluor 532. It is fully compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum*	525 nm
Emission maximum*	547 nm
Extinction coefficient (260 nm)	28,600 $M^{-1}cm^{-1}$
Extinction coefficient (525 nm)	86,500 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.57
Hydrophobicity by C18 chromatography***	25.6 (moderately hydrophobic)
pH dependence of dye fluorescence	Independent above pH 7 (see Appendix C)



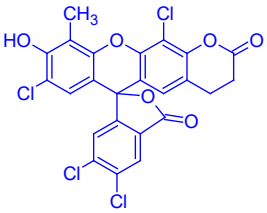


Available Yakima Yellow products	Product number
Yakima Yellow Lactone	M830684
Yakima Yellow Phosphoramidite	M830552
Yakima Yellow CPG	M830395
Yakima Yellow Polystyrene****	M400063 (bulk), M100443 (columns)

**Measured for T_8-YY conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-YY conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-YY Dye Calibrator (M830730) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

Yakima Yellow Lactone

Product number: M830684; CAS #: 502484-58-4



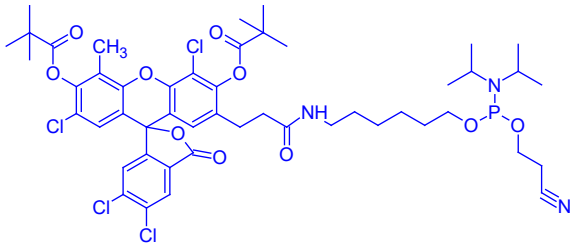
Formula	$C_{24}H_{12}Cl_4O_6$
Molecular weight (reagent)	538.16 Da
Molecular weight (incorporated)	538.16 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830684	bulk
M830684-1MG	1 mg
M830684-10MG	10 mg

Yakima Yellow Phosphoramidite

Product number: M830552; CAS #: 502485-39-4

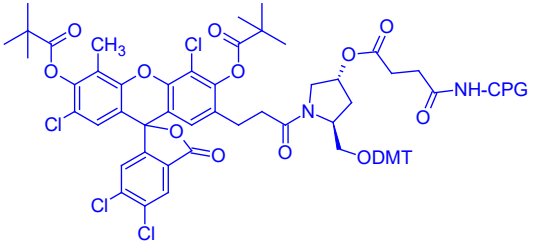


Formula	$C_{49}H_{60}Cl_4N_3O_{10}P$
Molecular weight (reagent)	1023.81 Da
Molecular weight (incorporated)	717.31 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830552	bulk
M830552-50UMOL	50 µmol
M830552-100UMOL	100 µmol
M830552-250MG	250 mg
M830552-1000MG	1 g

Yakima Yellow CPG

Product number: M830395

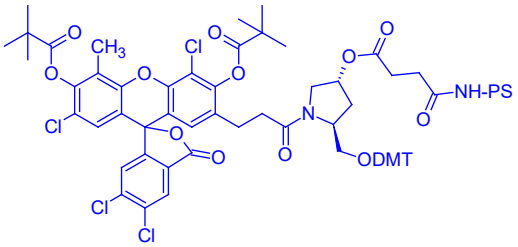


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	717.27 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 8 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830395	bulk
M830395-100MG	100 mg
M830395-250MG	250 mg
M830395-1000MG	1 g

Yakima Yellow Polystyrene

Product numbers: M400063 (bulk), M100443 (columns)



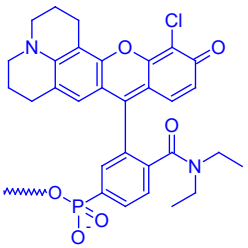
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	717.27 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M400063	bulk
M100443-P3	Pack of 3 columns*
M100443-P10	Pack of 10 columns*
M100443-P30	Pack of 30 columns*
M100443-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

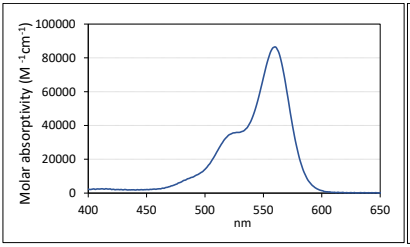
AquaPhluor 559

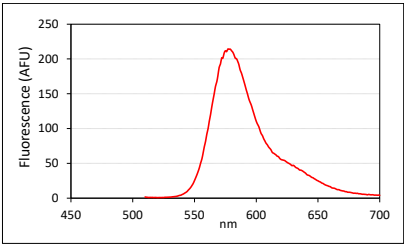
Acronym: AP559



AquaPhluor 559 is a fluorescent dye with excitation and emission properties similar to TAMRA and NED. The dye is compatible with oligonucleotide synthesis and deprotection.

Absorbance maximum*	559 nm
Emission maximum*	581 nm
Extinction coefficient (260 nm)	28,300 $M^{-1}cm^{-1}$
Extinction coefficient (559 nm)	86,500 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.64
Hydrophobicity by C18 chromatography***	25.6 (moderately hydrophobic)
pH dependence of dye fluorescence	Weakly dependent between pH of 5 and 8 (see Appendix C)



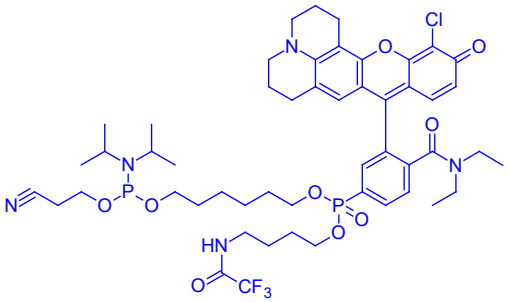


Available AquaPhluor 559 products	Product number
AquaPhluor 559 Internal Phosphoramidite	M830195
AquaPhluor 559 Terminal Phosphoramidite	M830698
AquaPhluor 559 CPG	M830673
AquaPhluor 559 Polystyrene****	M830674 (bulk), M100278 (columns)

**Measured for T_8-AP559 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP559 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP559 Dye Calibrator (M301430) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 559 Terminal Phosphoramidite

Product number: M830698; CAS #: 2378004-43-2

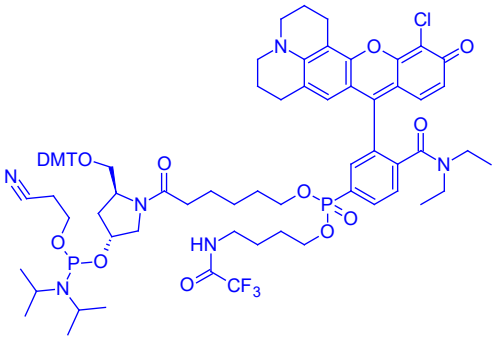


Formula	$C_{51}H_{67}ClF_3N_5O_9P_2$
Molecular weight (reagent)	1048.50 Da
Molecular weight (incorporated)	743.11 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830698	bulk
M830698-50UMOL	50 µmol
M830698-100UMOL	100 µmol
M830698-250MG	250 mg
M830698-1000MG	1 g

AquaPhluor 559 Internal Phosphoramidite

Product number: M830195; CAS #: 2378004-38-5

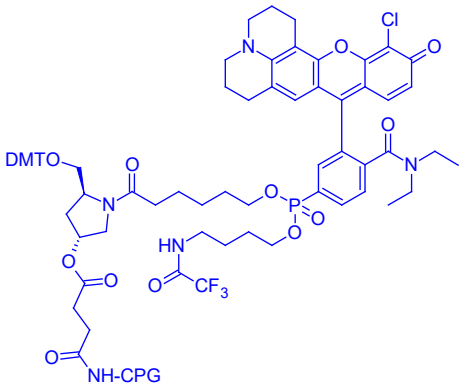


Formula	$C_{77}H_{92}ClF_3N_6O_{13}P_2$
Molecular weight (reagent)	1463.98 Da
Molecular weight (incorporated)	856.23 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥50%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830195	bulk
M830195-50UMOL	50 µmol
M830195-100UMOL	100 µmol
M830195-250MG	250 mg
M830195-1000MG	1 g

AquaPhluor 559 CPG

Product number: M830673

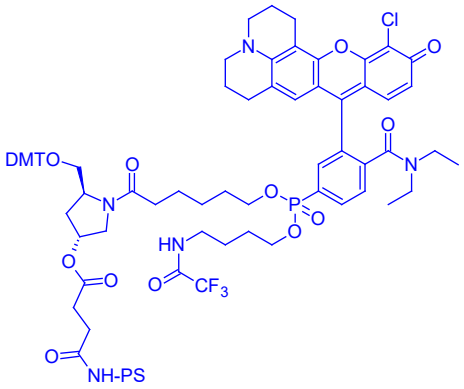


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	856.23 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Coupling efficiency test	N/A
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830673	bulk
M830673-100MG	100 mg
M830673-250MG	250 mg
M830673-1000MG	1 g

AquaPhluor 559 Polystyrene

Product numbers: M830674 (bulk), M100278 (columns)



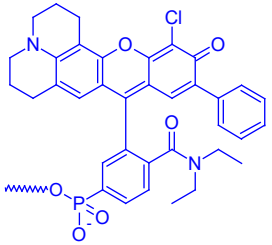
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	856.23 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830674	bulk
M100278-P3	Pack of 3 columns*
M100278-P10	Pack of 10 columns*
M100278-P30	Pack of 30 columns*
M100278-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

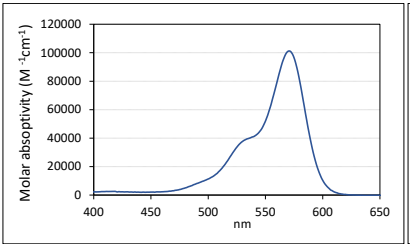
AquaPhluor 570

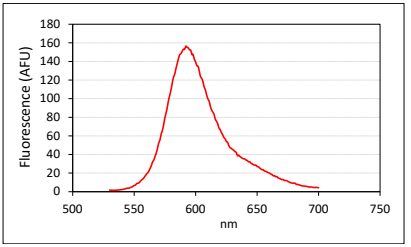
Acronym: AP570



AquaPhluor 570 is a fluorescent dye with excitation and emission properties similar to Redmond Red. Unlike Redmond Red it is pH insensitive and fully compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum*	570 nm
Emission maximum*	592 nm
Extinction coefficient (260 nm)	44,100 $M^{-1}cm^{-1}$
Extinction coefficient (570 nm)	101,100 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.68
Hydrophobicity by C18 chromatography***	29.4 (hydrophobic)
pH dependence of dye fluorescence	Weakly dependent between pH 5 and 8 (Appendix C)



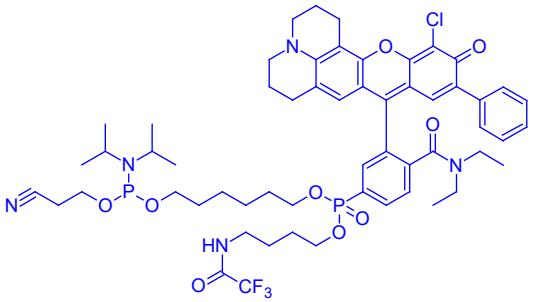


Available AquaPhluor 570 products	Product number
AquaPhluor 570 Internal Phosphoramidite	M830675
AquaPhluor 570 Terminal Phosphoramidite	M830699
AquaPhluor 570 CPG	M830676
AquaPhluor 570 Polystyrene****	M830677 (bulk), M100444 (columns)

**Measured for T_8-AP570 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP570 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP570 Dye Calibrator (M830700) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 570 Terminal Phosphoramidite

Product number: M830699; CAS #: 2378004-30-7

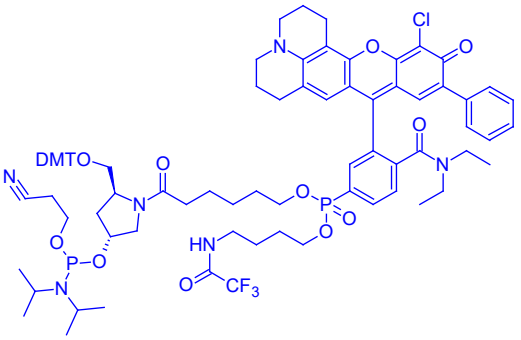


Formula	$C_{57}H_{71}ClF_3N_5O_9P_2$
Molecular weight (reagent)	1124.59 Da
Molecular weight (incorporated)	819.2 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830699	bulk
M830699-50UMOL	50 µmol
M830699-100UMOL	100 µmol
M830699-250MG	250 mg
M830699-1000MG	1 g

AquaPhluor 570 Internal Phosphoramidite

Product number: M830675; CAS #: 2378004-20-5

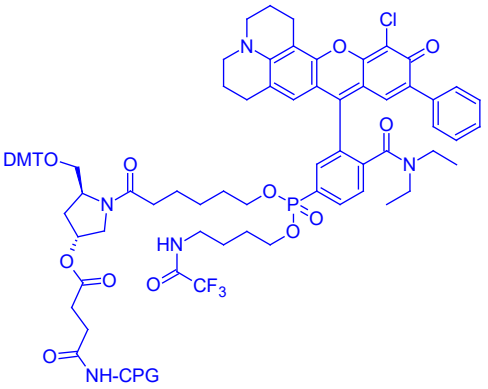


Formula	$C_{83}H_{96}ClF_3N_6O_{13}P_2$
Molecular weight (reagent)	1540.08 Da
Molecular weight (incorporated)	932.33 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830675	bulk
M830675-50UMOL	50 µmol
M830675-100UMOL	100 µmol
M830675-250MG	250 mg
M830675-1000MG	1 g

AquaPhluor 570 CPG

Product number: M830676

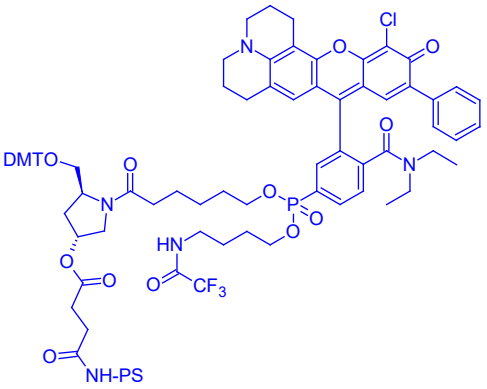


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	932.33 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830676	bulk
M830676-100MG	100 mg
M830676-250MG	250 mg
M830676-1000MG	1 g

AquaPhluor 570 Polystyrene

Product numbers: M830677 (bulk), M100444 (columns)



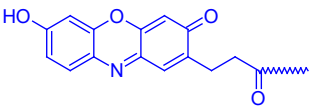
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	932.33 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830677	bulk
M100444-P3	Pack of 3 columns*
M100444-P10	Pack of 10 columns*
M100444-P30	Pack of 30 columns*
M100444-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

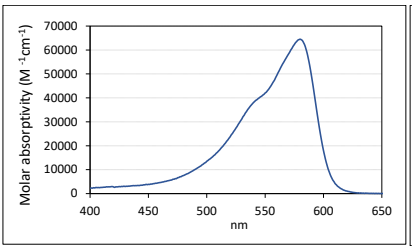
Redmond Red®

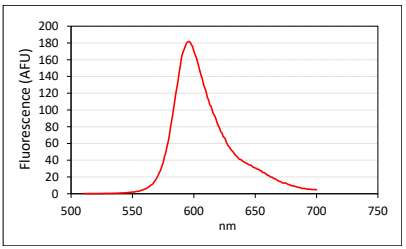
Acronym: RR



Redmond Red is a fluorescent dye with excitation and emission properties similar to those of AquaPhluor 570. It is compatible with oligonucleotide synthesis. Mild deprotection conditions are recommended.

Absorbance maximum*	580 nm
Emission maximum*	594 nm
Extinction coefficient (260 nm)	9,800 $M^{-1}cm^{-1}$
Extinction coefficient (580 nm)	64,500 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.46
Hydrophobicity by C18 chromatography***	17.9 (hydrophilic)
pH dependence of dye fluorescence	Highly pH-dependent, pK_a ~7 (see Appendix C)



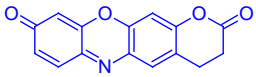


Available Redmond Red products	Product number
Redmond Red Lactone	M830728
Redmond Red Phosphoramidite	M830035
Redmond Red CPG	M830390
Redmond Red Polystyrene****	M830729 (bulk), M100445 (columns)

**Measured for T_8-RR conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-RR conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-RR Dye Calibrator (M830731) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

Redmond Red Lactone

Product number: M830728; CAS #: 502484-16-4



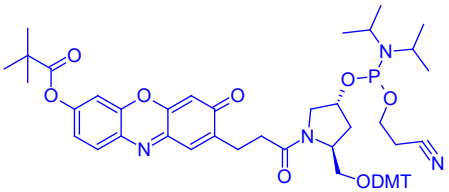
Formula	$C_{15}H_9NO_4$
Molecular weight (reagent)	267.24 Da
Molecular weight (incorporated)	267.24 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830728	bulk
M830728-1MG	1 mg
M830728-10MG	10 mg

Redmond Red Phosphoramidite

Product number: M830035; CAS #: 909906-38-3

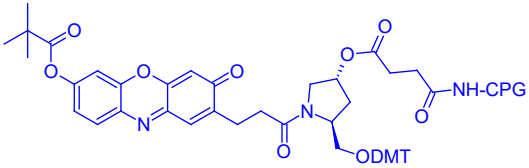


Formula	$C_{55}H_{63}N_4O_{10}P$
Molecular weight (reagent)	971.08 Da
Molecular weight (incorporated)	446.34 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830035	bulk
M830035-50UMOL	50 µmol
M830035-100UMOL	100 µmol
M830035-250MG	250 mg
M830035-1000MG	1 g

Redmond Red CPG

Product number: M830390

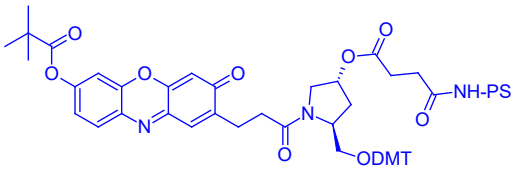


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	446.34 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830390	bulk
M830390-100MG	100 mg
M830390-250MG	250 mg
M830390-1000MG	1 g

Redmond Red Polystyrene

Product numbers: M830729 (bulk), M100445 (columns)



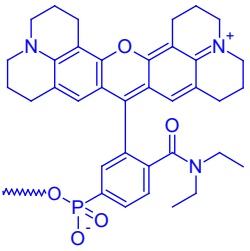
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	446.34 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Coupling efficiency test	N/A
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830729	bulk
M100445-P3	Pack of 3 columns*
M100445-P10	Pack of 10 columns*
M100445-P30	Pack of 30 columns*
M100445-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

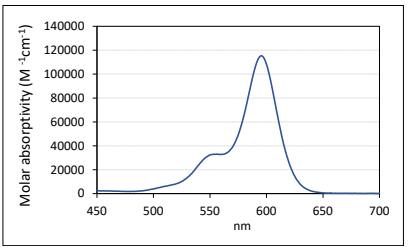
AquaPhluor 593

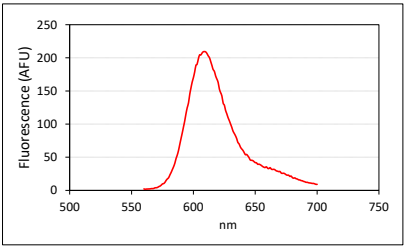
Acronym: AP593



AquaPhluor 593 is a fluorescent dye with excitation and emission properties similar to ROX and Texas Red. The dye is stable under the standard oligonucleotide synthesis and deprotection conditions.

Absorbance maximum*	593 nm
Emission maximum*	613 nm
Extinction coefficient (260 nm)	19,680 $M^{-1}cm^{-1}$
Extinction coefficient (593 nm)	115,240 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.61
Hydrophobicity by C18 chromatography***	29.9 (hydrophobic)
pH dependence of dye fluorescence	Weakly dependent between pH 5 and 8 (Appendix C)



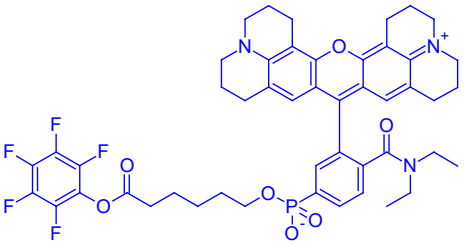


Available AquaPhluor 593 products	Product number
AquaPhluor 593 PFP ester	M830196
AquaPhluor 593 Terminal Phosphoramidite	M830736
AquaPhluor 593 CPG	M830208
AquaPhluor 593 Polystyrene****	M830732 (bulk), M100257 (columns)

**Measured for T_8-AP593 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP593 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP593 Dye Calibrator (M300392) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 593 PFP ester

Product number: M830196; CAS #: 1140966-99-9



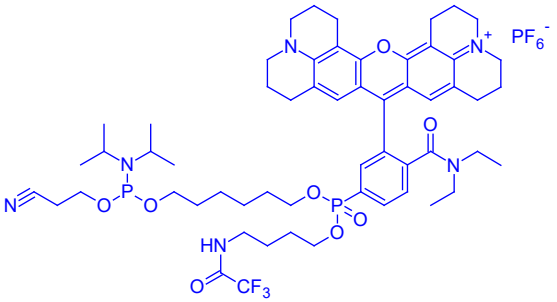
Formula	$C_{48}H_{49}F_5N_3O_7P$
Molecular weight (reagent)	905.88 Da
Molecular weight (incorporated)	721.82 Da
Purity* (reverse phase HPLC) @590 nm	≥70%
Structure identity test	^{1}H NMR
Recommended coupling conditions:	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 3 years
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 70%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830196	bulk
M830196-1MG	1 mg
M830196-10MG	10 mg

AquaPhluor 593 Terminal Phosphoramidite

Product number: M830736; CAS #: 2378004-17-0

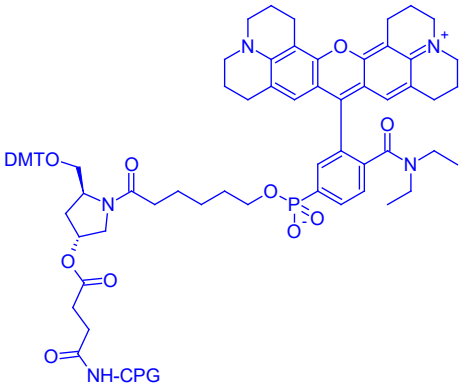


Formula	$C_{57}H_{78}F_9N_6O_8P_3$
Molecular weight (reagent)	1239.17 Da
Molecular weight (incorporated)	787.8 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830736	bulk
M830736-50UMOL	50 µmol
M830736-100UMOL	100 µmol
M830736-250MG	250 mg
M830736-1000MG	1 g

AquaPhluor 593 CPG

Product number: M830208

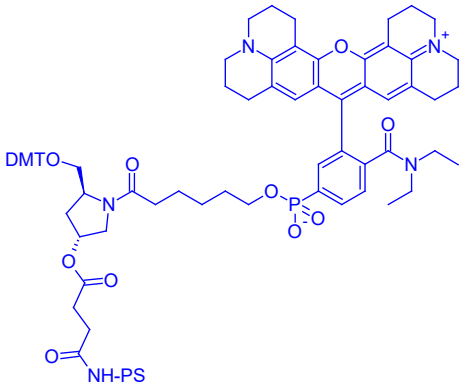


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	900.93 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥80%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830208	bulk
M830208-100MG	100 mg
M830208-250MG	250 mg
M830208-1000MG	1 g

AquaPhluor 593 Polystyrene

Product numbers: M830732 (bulk), M100257 (columns)



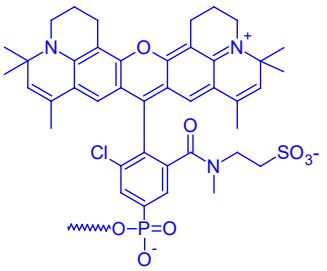
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	900.93 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥80%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 7 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830732	bulk
M100257-P3	Pack of 3 columns*
M100257-P10	Pack of 10 columns*
M100257-P30	Pack of 30 columns*
M100257-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

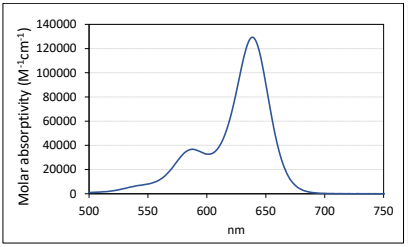
AquaPhluor 639

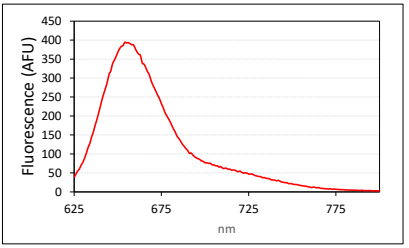
Acronym: AP639



AquaPhluor 639 is a fluorescent dye with excitation and emission properties similar to Cy5, Quasar 670 and AP642. It features sequence-independent absorption and fluorescence spectra and excellent chemical and photo-chemical stability. AP639 is compatible with both standard and mild oligonucleotide deprotection conditions.

Absorbance maximum*	639 nm
Emission maximum*	655 nm
Extinction coefficient (260 nm)	24,650 $M^{-1}cm^{-1}$
Extinction coefficient (639 nm)	129,300 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.28
Hydrophobicity by C18 chromatography***	37.5 (hydrophobic)
pH dependence of dye fluorescence	Independent between pH 5 and 8 (Appendix C)



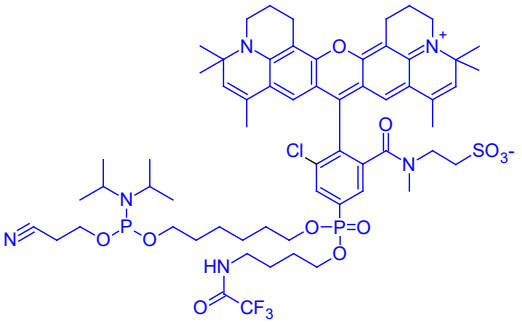


Available AquaPhluor 639 products	Product number
AquaPhluor 639 Terminal Phosphoramidite	M830741
AquaPhluor 639 CPG	M830757
AquaPhluor 639 Polystyrene	M830743 (bulk), M100440 (columns****)

**Measured for T_8-AP639 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP639 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP639 Dye Calibrator (M301715) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 639 Terminal Phosphoramidite

Product number: M830741; CAS #: 2378724-63-9

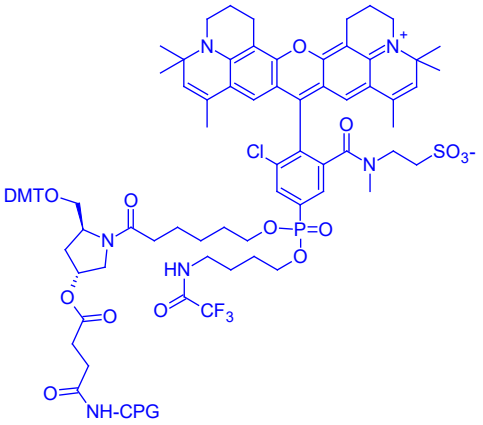


Formula	$C_{62}H_{82}ClF_3N_6O_{11}P_2S$
Molecular weight (reagent)	1273.81 Da
Molecular weight (incorporated)	968.42 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830741	bulk
M830741-50UMOL	50 µmol
M830741-100UMOL	100 µmol
M830741-250MG	250 mg
M830741-1000MG	1 g

AquaPhluor 639 CPG

Product numbers: M830757

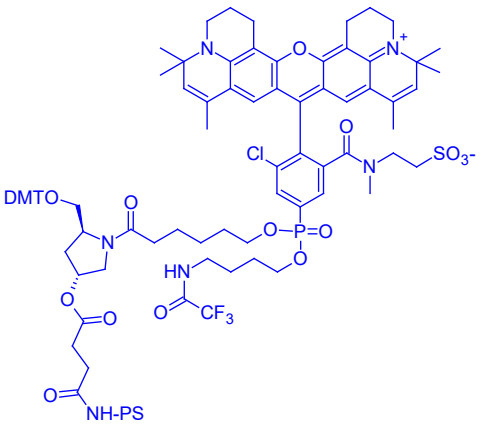


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1081.54 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830757	bulk
M830757-100MG	100 mg
M830757-250MG	250 mg
M830757-1000MG	1 g

AquaPhluor 639 Polystyrene

Product numbers: M830743 (bulk), M100440 (columns)



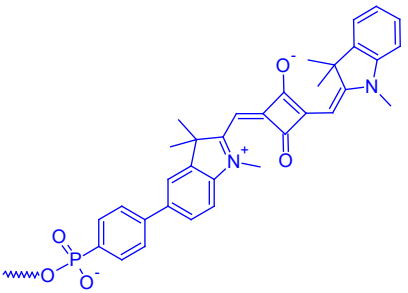
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1081.54 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥70%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830743	bulk
M100440-P3	Pack of 3 columns*
M100440-P10	Pack of 10 columns*
M100440-P30	Pack of 30 columns*
M100440-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

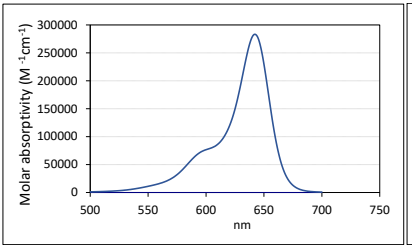
AquaPhluor 642

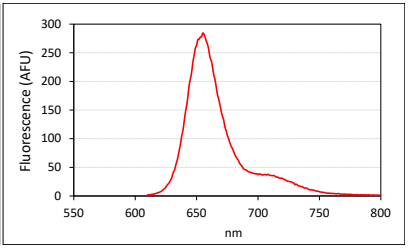
Acronym: AP642



AquaPhluor 642 is a fluorescent dye with excitation and emission properties similar to Cy5. Mild deprotection conditions are recommended for products carrying this dye.

Absorbance maximum*	641 nm
Emission maximum*	653 nm
Extinction coefficient (260 nm)	19,700 $M^{-1}cm^{-1}$
Extinction coefficient (641 nm)	283,200 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.13
Hydrophobicity by C18 chromatography***	33.9 (hydrophobic)
pH dependence of dye fluorescence	Independent between pH 5 and 8 (Appendix C)



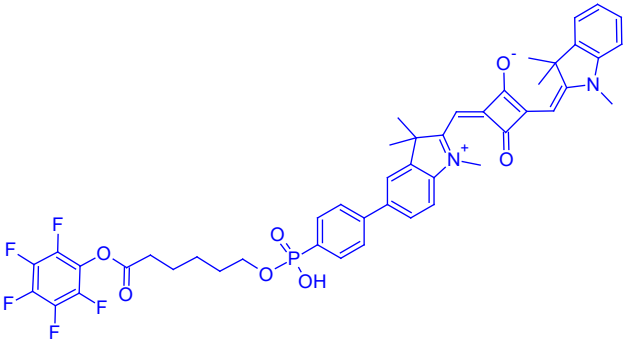


Available AquaPhluor 642 products	Product number
AquaPhluor 642 PFP ester	M830197
AquaPhluor 642 CPG	M830190
AquaPhluor 642 Polystyrene****	M830678 (bulk), M100279 (columns)

**Measured for T_8-AP642 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP642 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP642 Dye Calibrator (M300589) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 642 PFP ester

Product number: M830197; CAS #: 2378043-93-5



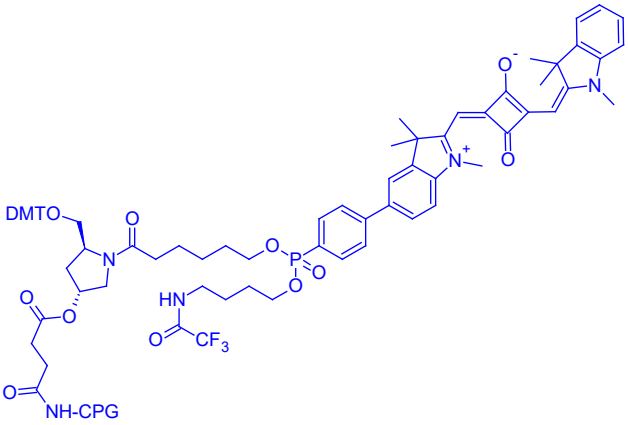
Formula	$C_{46}H_{42}F_5N_2O_7P$ (zwitterionic form)
Molecular weight (reagent)	860.80 Da
Molecular weight (incorporated)	676.73 Da
Purity* (reverse phase HPLC)	≥80%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 80%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830197	bulk
M830197-1MG	1 mg
M830197-10MG	10 mg

AquaPhluor 642 CPG

Product number: M830190

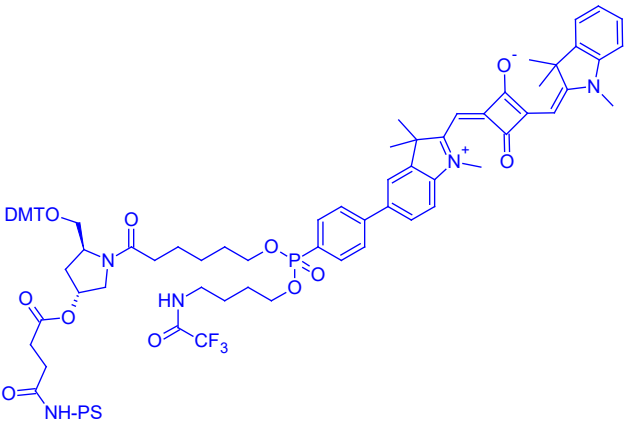


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	855.84 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥70%
Coupling efficiency test	N/A
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830190	bulk
M830190-100MG	100 mg
M830190-250MG	250 mg
M830190-1000MG	1 g

AquaPhluor 642 Polystyrene

Product numbers: M830678 (bulk), M100280 (200 nmol column), M100279 (1 µmol column)



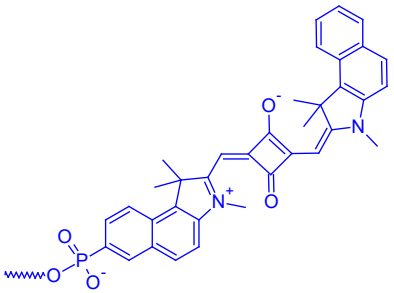
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	855.84 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥70%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830678	bulk
M100280-P3	Pack of 3 columns*
M100280-P10	Pack of 10 columns*
M100280-P30	Pack of 30 columns*
M100280-P100	Pack of 100 columns*
M100279-P3	Pack of 3 columns*
M100279-P10	Pack of 10 columns*
M100279-P30	Pack of 30 columns*
M100279-P100	Pack of 100 columns*

**ABI 3900 type columns*

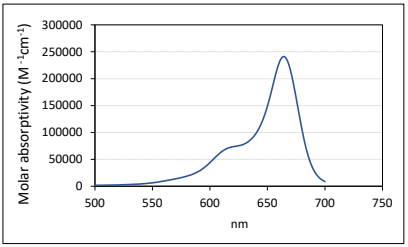
AquaPhluor 662

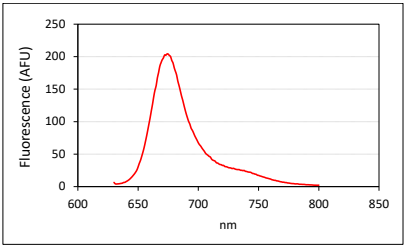
Acronym: AP662



AquaPhluor 662 is a fluorescent dye with excitation and emission properties similar to those of Cy5. Mild deprotection conditions are recommended for products carrying this dye.

Absorbance maximum*	662 nm
Emission maximum*	672 nm
Extinction coefficient (260 nm)	15,600 $M^{-1}cm^{-1}$
Extinction coefficient (662 nm)	241,100 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.14
Hydrophobicity by C18 chromatography***	35.0 (hydrophobic)
pH dependence of dye fluorescence	Independent between pH 5 and 8 (Appendix C)





Available AquaPhluor 662 products	Product number
AquaPhluor 662 PFP ester	M830198
AquaPhluor 662 CPG	M830191
AquaPhluor 662 Polystyrene****	M830679 (bulk), M100271 (columns)

**Measured for T_8-AP662 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

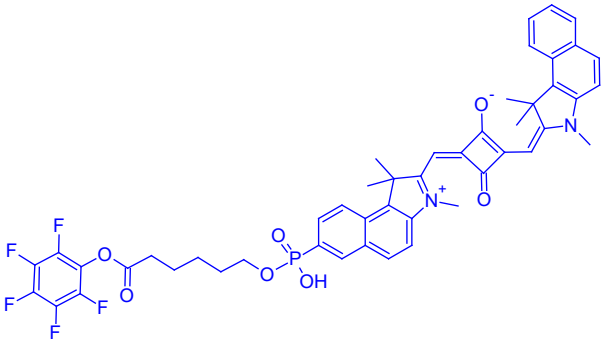
***Measured for T_8-AP662 conjugate (0.1 M Sodium tetraborate)*

****Percent acetonitrile required for elution of T_8-AP662 Dye Calibrator (M830724) from C18 column (see Appendix A for details)*

*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 662 PFP ester

Product number: M830198; CAS #: 2378043-92-4



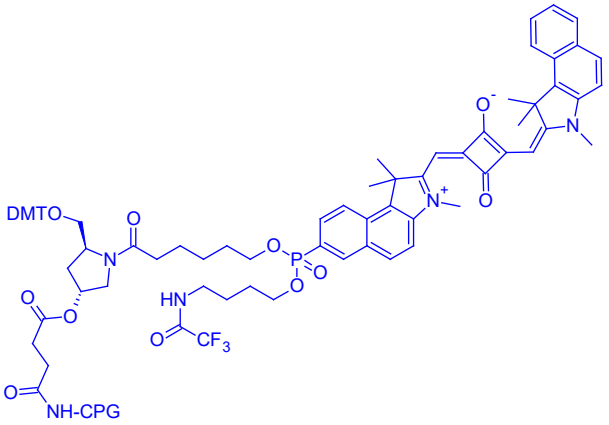
Formula	$C_{48}H_{42}F_5N_2O_7P$ (zwitterionic form)
Molecular weight (reagent)	884.82 Da
Molecular weight (incorporated)	700.75 Da
Purity* (reverse phase HPLC)@640 nm	≥85%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 85%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830198	bulk
M830198-1MG	1 mg
M830198-10MG	10 mg

AquaPhluor 662 CPG

Product number: M830191

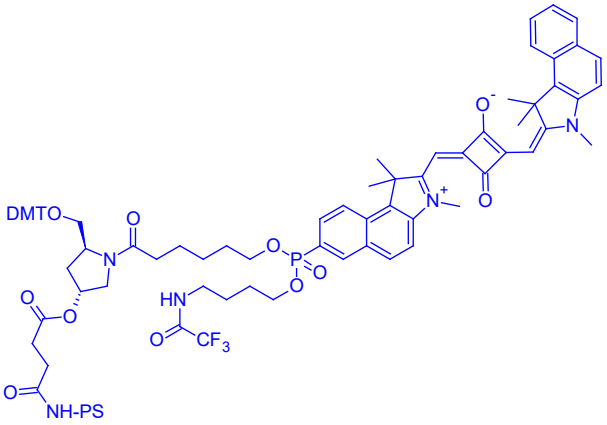


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	879.86 Da
Capacity by DMT loading	≥ 15µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830191	bulk
M830191-100MG	100 mg
M830191-250MG	250 mg
M830191-1000MG	1 g

AquaPhluor 662 Polystyrene

Product numbers: M830679 (bulk), M100271 (columns)



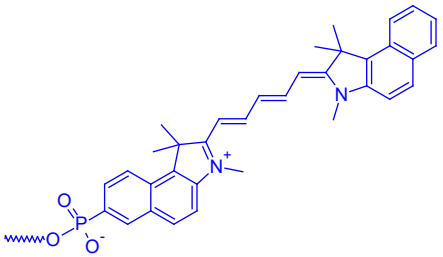
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	879.86 Da
Capacity by DMT loading	≥8 µmol/g
Purity of test oligo (reverse phase HPLC)	≥80%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830679	bulk
M100271-P3	Pack of 3 columns*
M100271-P10	Pack of 10 columns*
M100271-P30	Pack of 30 columns*
M100271-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

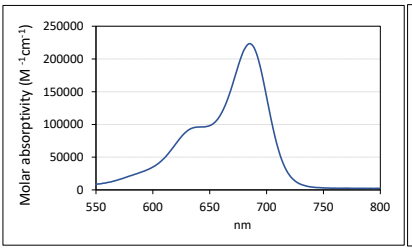
AquaPhluor 680

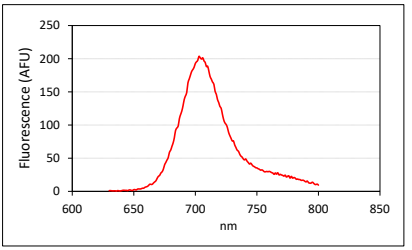
Acronym: AP680



AquaPhluor 680 is a fluorescent dye with excitation and emission properties similar to those of Cy5.5. Mild deprotection conditions are recommended for products carrying this dye.

Absorbance maximum*	682 nm
Emission maximum*	704 nm
Extinction coefficient (260 nm)	16,100$M^{-1}cm^{-1}$
Extinction coefficient (682 nm)	221,300 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.07
Hydrophobicity by C18 chromatography***	35.8** (hydrophobic)
pH dependence of dye fluorescence	Independent between pH 5 & 8 (Appendix C)



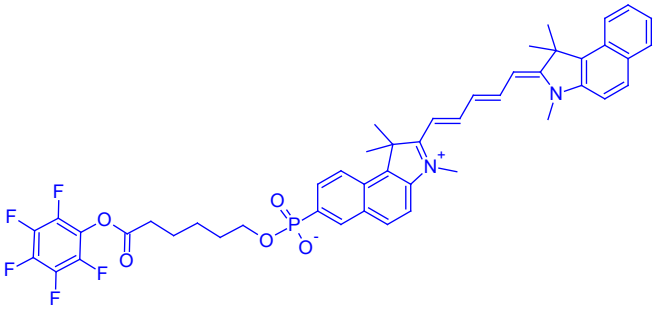


Available AquaPhluor 680 products	Product number
AquaPhluor 680 PFP ester	M830682
AquaPhluor 680 CPG	M830680
AquaPhluor 680 Polystyrene****	M830681 (bulk), M100282 (columns)

**Measured for T_8-AP680 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP680 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP680 Dye Calibrator (M300753) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 680 PFP ester

Product number: M830682; CAS #: 2378004-18-1



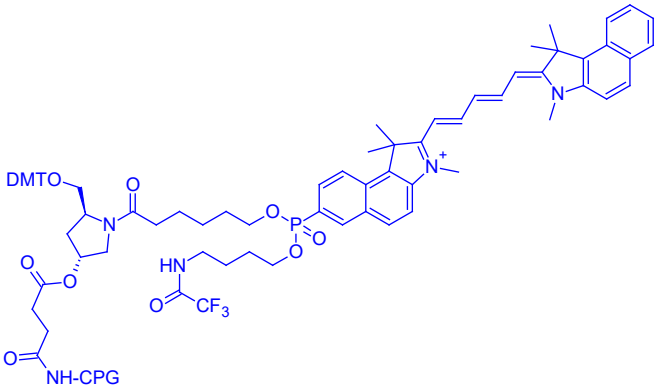
Formula	$C_{47}H_{44}F_5N_2O_5P$ (zwitterionic form)
Molecular weight (reagent)	842.83 Da
Molecular weight (incorporated)	658.76 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830682	bulk
M830682-1MG	1 mg
M830682-10MG	10 mg

AquaPhluor 680 CPG

Product number: M830680

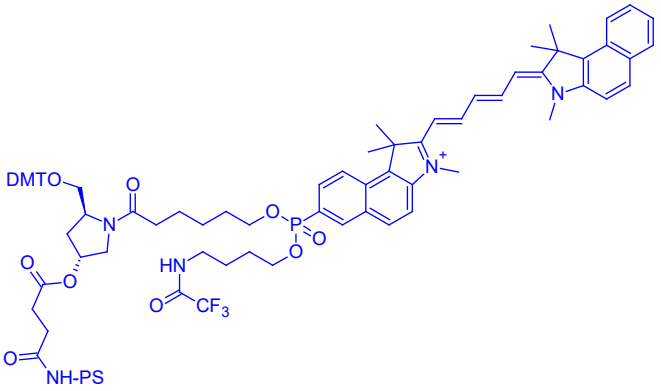


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	837.88 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1), 8 h at 50°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830680	bulk
M830680-100MG	100 mg
M830680-250MG	250 mg
M830680-1000MG	1 g

AquaPhluor 680 Polystyrene

Product numbers: M830681 (bulk), M100282 (columns)



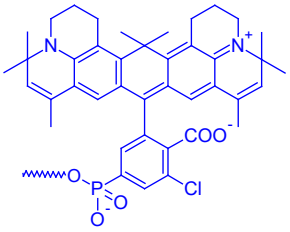
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	837.88 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1), 8 h @ 50°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830681	bulk
M100282-P3	Pack of 3 columns*
M100282-P10	Pack of 10 columns*
M100282-P30	Pack of 30 columns*
M100282-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

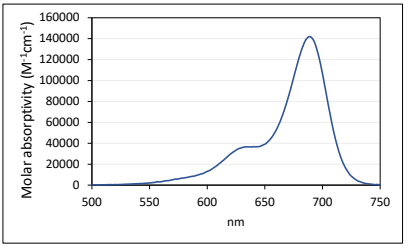
AquaPhluor 690

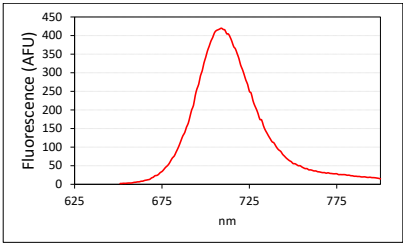
Acronym: AP690



AquaPhluor 690 is a fluorescent dye with excitation and emission properties similar to AP680, Cy5.5 and Quasar 705. It features sequence-independent absorption and fluorescence spectra and improved chemical and photo-chemical stability as compared to the spectrally-similar AquaPhluor 680, Cy5.5 and Quasar 705 dyes.

Absorbance maximum*	689 nm
Emission maximum*	709 nm
Extinction coefficient (260 nm)	23,500 $M^{-1}cm^{-1}$
Extinction coefficient (689 nm)	141,900 $M^{-1}cm^{-1}$
Fluorescence quantum yield**(Φ)	0.14
Hydrophobicity by C18 chromatography***	30.5 (hydrophobic)
pH dependence of dye fluorescence	Independent between pH 5 & 8 (Appendix C)



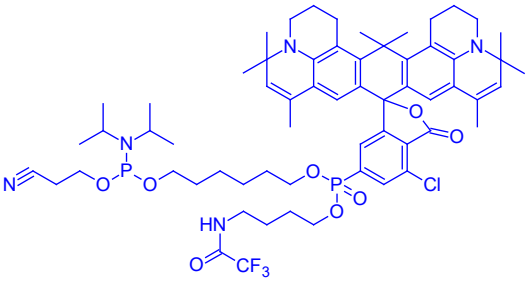


Available AquaPhluor 690 products	Product number
AquaPhluor 690 Terminal Phosphoramidite	M830760
AquaPhluor 690 CPG	M830759
AquaPhluor 690 Polystyrene****	M830755 (bulk), M100500 (columns)

**Measured for T_8-AP690 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for T_8-AP690 conjugate (0.1 M Sodium tetraborate)*
****Percent acetonitrile required for elution of T_8-AP690 Dye Calibrator (M302123) from C18 column (see Appendix A for details)*
*****Suitable for ABI 3900 DNA synthesizer*

AquaPhluor 690 Terminal Phosphoramidite

Product number: M830760; CAS #: 2378004-21-6

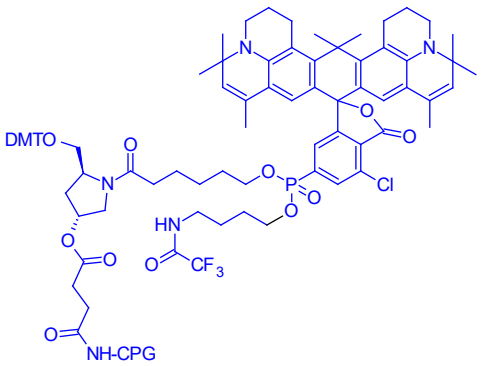


Formula	$C_{62}H_{81}ClF_3N_5O_8P_2$
Molecular weight (reagent)	1178.73 Da
Molecular weight (incorporated)	873.34 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830760	bulk
M830760-50UMOL	50 µmol
M830760-100UMOL	100 µmol
M830760-250MG	250 mg
M830760-1000MG	1 g

AquaPhluor 690 CPG

Product numbers: M830759

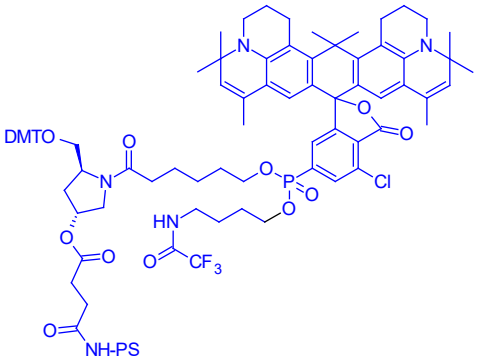


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	986.46 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥70%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830759	bulk
M830759-100MG	100 mg
M830759-250MG	250 mg
M830759-1000MG	1 g

AquaPhluor 690 Polystyrene

Product numbers: M830755 (bulk), M100500 (columns)



Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	986.46 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830755	bulk
M100500-P3	Pack of 3 columns*
M100500-P10	Pack of 10 columns*
M100500-P30	Pack of 30 columns*
M100500-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

3. Fluorescence Quenchers

Azo Dye Quenchers

ELITechGroup's azo dye quenchers have absorption spectra covering fluorophores with emissions from 400 nm to 750 nm (Figure 5). They have been designed with the relatively long range (20-60 Å) fluorescence resonance transfer (FRET) quenching mechanism in mind, which requires a spectral overlap between fluorophore emission and quencher absorption spectra.

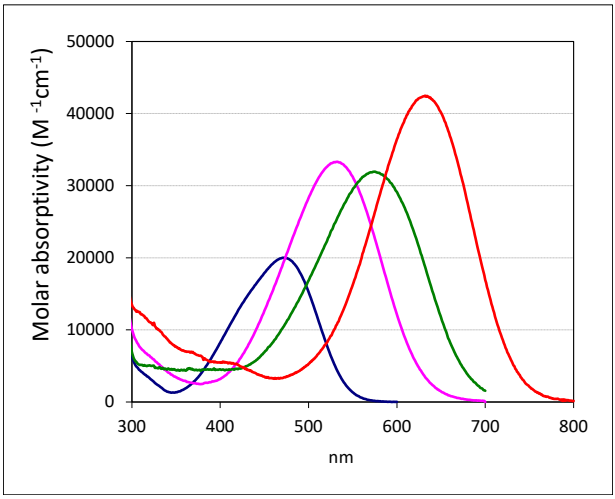


Figure 5 Absorption spectra of azo dye quenchers: Quencher-470 (blue), Eclipse® Dark Quencher (pink), Quencher-575 (green), Quencher-630 (red).

Azo dyes are also known to exert non-FRET quenching, so-called contact quenching. Contact quenching (also known as quenching by 'touching' or collisional quenching) is not based on the long range FRET mechanism and requires close contact between fluorophore and quencher. This mechanism works especially well in Molecular Beacons wherein 'touching' is enhanced by the formation of the self-complementary double-stranded stem.

Based on the dynamics of fluorophore-quencher interactions, quenching was categorized as being either dynamic or static in complex formation (Ref. 4). Three different dynamic contact quenching mechanisms were identified: intersystem crossing, electron exchange, and photoinduced electron transfer (PET). At least the last two mechanisms have been shown to be present in known nucleic acid probes. For example, the electron

exchange (also known as Dexter interaction) is present (along with FRET) in linear dual labeled probes (such as TaqMan) and requires temporary orbital overlap. The photoinduced electron transfer between a fluorescent dye and one or more guanine bases, located in close proximity to the dye, is the quenching mechanism for DNA probes described in Ref. 5 and 6. Examples of PET-mediated quenching by nitroindole nucleosides are also described (Ref. 7). It has been shown (Ref. 8) that the $CDPI_3$-type MGB demonstrates the PET mechanism as well. An example of static quenching is described in Ref. 9; it is characterized by the formation of a ground state complex (hetero-dimer) between a fluorophore and an azo dye quencher accompanied by significant changes in absorption spectra of the dyes.

Contact quenching is present to a varying degree in other probe chemistries that do not have any intentional secondary structure (e.g., TaqMan). For such probes the contribution of contact quenching will generally diminish as the length of the probe increases.

In general, when selecting a quencher, one should consider which quenching mechanism is predominant for a given probe chemistry. For example, for longer non-MGB TaqMan probes, wherein FRET is expected to be the main quenching mechanism, a quencher with the best spectral overlap with fluorophore emission should be selected. On the other hand, for MGB TaqMan or MGB Pleiades probes, which are generally shorter and also benefit from the MGB-induced PET quenching, the Eclipse Dark Quencher will work well regardless of fluorophore emission spectrum.

Lite Quenchers™

Lite Quenchers (LQ-380 and LQ-400) are nitrodiarylethene (nitrostilbene) analogues (Ref. 10) whose absorption spectra are substantially blue-shifted relative to the emission spectra of common fluorophores (such as fluorescein). These so-called non-FRET quenchers do not rely on the spectral overlap of quencher absorbance and fluorophore emission (FRET mechanism) for their quenching abilities. They have been designed to eliminate (or significantly weaken) the FRET and preserve the contact quenching. As a result, the oligonucleotide probes possessing such quenchers have fluorescence characteristics that are not always achievable using traditional FRET quenchers.

Lite Quenchers are particularly useful in oligonucleotide hybridization probes having fewer than 12 bases between the fluorophore and quencher. Oligonucleotide probes of this length, when labeled with traditional FRET quenchers, demonstrate significantly reduced fluorescence signals upon

hybridization with their targets. The signal drop is due to insufficient spatial separation between the fluorophore and quencher leading to a residual FRET quenching. The Lite Quenchers, which have substantially diminished FRET quenching properties, avoid this disadvantage.

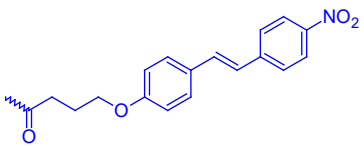
A particular class of probes that is suitable for use in conjunction with the Lite Quenchers are very short (8-12 bases long) MGB Pleiades probes. Such probes are short due to the duplex stabilizing effect of the MGB and are optimal for distinguishing closely related nucleic acid targets. Importantly, these probes containing the Lite Quenchers demonstrate low background fluorescence and high hybridization signals, the latter being significantly higher than those achieved using the traditional FRET-based quenchers. Both background fluorescence and signal are essential parameters for improving assay sensitivity. Such probes are useful, for instance, in digital PCR, such as described in Ref. 11.

Molecular Beacons are another type of probe that may demonstrate improved fluorescence characteristics when used with Lite Quenchers.

Azo dye and Lite Quenchers are available as 2-cyanoethyl dye phosphoramidites, dye-modified oligonucleotide synthesis solid supports for on-line dye incorporation, and amine-reactive esters for post-synthesis conjugation.

Lite Quencher-380

Acronym: LQ-380

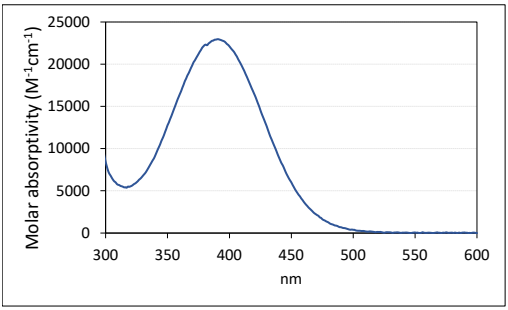


LQ-380 is a contact quencher with no spectral overlap with fluorophores emitting above 500 nm.

Products containing LQ-380 quencher are fully compatible with standard oligonucleotide synthesis and deprotection conditions.

Absorbance maximum*	388 nm
Extinction coefficient (260 nm)	9,300 $M^{-1}cm^{-1}$
Extinction coefficient (388 nm)	22,900 $M^{-1}cm^{-1}$

**Measured for T_8-LQ-380 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

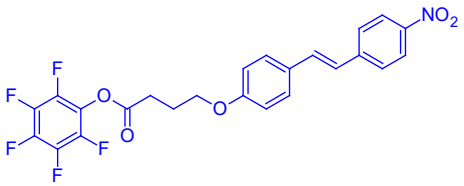


Available LQ-380 products	Product number
Lite Quencher-380 PFP ester	M830787
Lite Quencher-380 Internal Phosphoramidite	M830786
Lite Quencher-380 CPG	M830799
Lite Quencher-380 Polystyrene**	M830793 (bulk), M100505 (columns)

***Suitable for ABI 3900 DNA synthesizer*

Lite Quencher-380 PFP ester

Product number: M830787; CAS #: 2244400-57-3



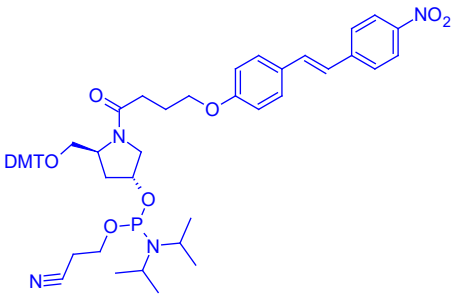
Formula	$C_{24}H_{16}F_5NO_5$
Molecular weight (reagent)	493.38 Da
Molecular weight (incorporated)	309.31 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830787	bulk
M830787-1MG	1 mg
M830787-10MG	10 mg

Lite Quencher-380 Internal Phosphoramidite

Product number: M830786; CAS #: 2244400-74-4

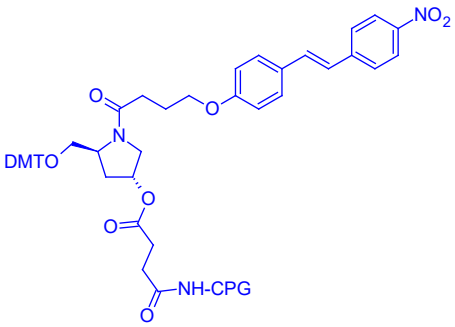


Formula	$C_{53}H_{61}N_4O_9PP$
Molecular weight (reagent)	929.05 Da
Molecular weight (incorporated)	488.42 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling condition	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830786	bulk
M830786-50UMOL	50 µmol
M830786-100UMOL	100 µmol
M830786-250MG	250 mg
M830786-1000MG	1 g

Lite Quencher-380 CPG

Product number: M830799

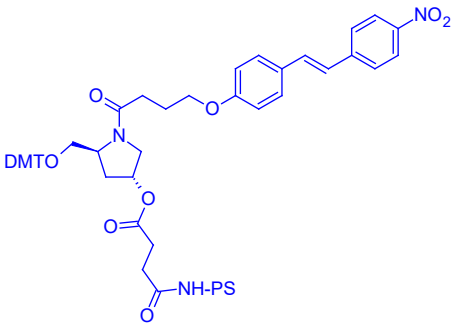


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	488.42 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830799	bulk
M830799-100MG	100 mg
M830799-250MG	250 mg
M830799-1000MG	1 g

Lite Quencher-380 Polystyrene

Product numbers: M830793 (bulk), M100505 (columns)



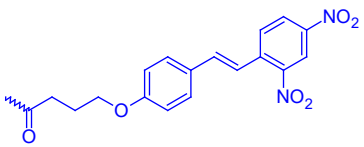
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	488.42 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Coupling efficiency test	N/A
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830793	bulk
M100505-P3	Pack of 3 columns*
M100505-P10	Pack of 10 columns*
M100505-P30	Pack of 30 columns*
M100505-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Lite Quencher-400

Acronym: LQ-400

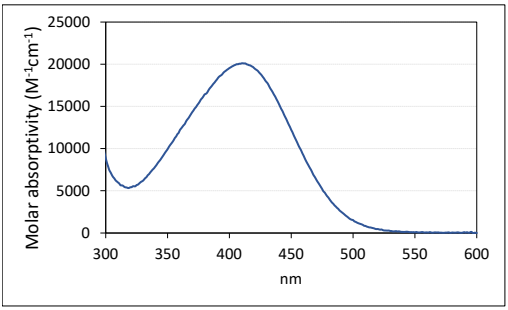


LQ-400 is a contact quencher with a minimal spectral overlap with the emission of fluorescein and no-overlap with fluorophores emitting above 525 nm.

Mild deprotection conditions are recommended for products containing LQ-400 quencher.

Absorbance maximum*	407 nm
Extinction coefficient (260 nm)	17,300 $M^{-1}cm^{-1}$
Extinction coefficient (407 nm)	20,000 $M^{-1}cm^{-1}$

**Measured for T_8-LQ-400 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

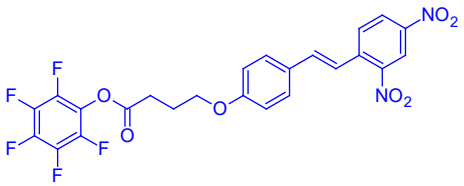


Available LQ-400 products	Product number
Lite Quencher-400 PFP ester	M830789
Lite Quencher-400 Internal Phosphoramidite	M830788
Lite Quencher-400 CPG	M830800
Lite Quencher-400 Polystyrene**	M830794 (bulk), M100507 (columns)

***Suitable for ABI 3900 DNA synthesizer*

Lite Quencher-400 PFP ester

Product number: M830789; CAS #: 2244400-58-4



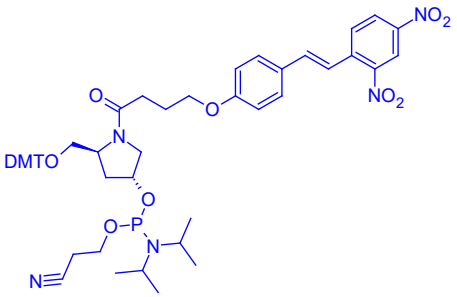
Formula	$C_{24}H_{15}F_5N_2O_7$
Molecular weight (reagent)	538.38 Da
Molecular weight (incorporated)	354.31 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830789	bulk
M830789-1MG	1 mg
M830789-10MG	10 mg

Lite Quencher-400 Internal Phosphoramidite

Product number: M830788; CAS #: 2244400-75-5

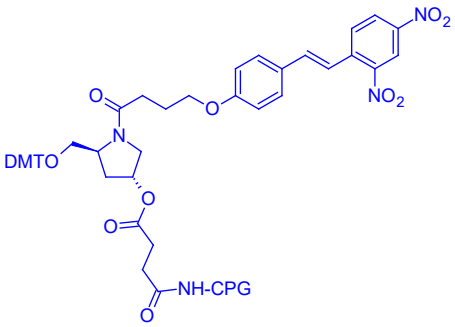


Formula	$C_{53}H_{60}N_5O_{11}P$
Molecular weight (reagent)	974.04 Da
Molecular weight (incorporated)	533.42 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling condition	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830788	bulk
M830788-50UMOL	50 µmol
M830788-100UMOL	100 µmol
M830788-250MG	250 mg
M830788-1000MG	1 g

Lite Quencher-400 CPG

Product number: M830800

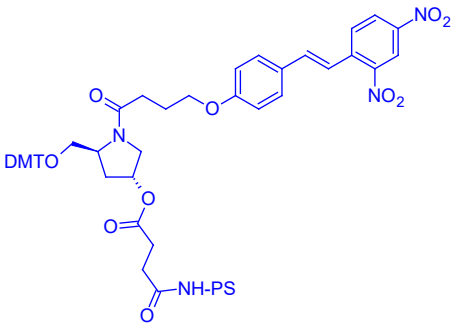


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	533.42 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830800	bulk
M830800-100MG	100 mg
M830800-250MG	250 mg
M830800-1000MG	1 g

Lite Quencher-400 Polystyrene

Product number: M830794 (bulk), M100507 (columns)



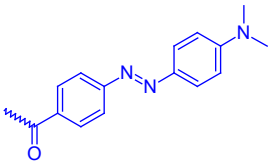
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	533.42 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830794	bulk
M100507-P3	Pack of 3 columns*
M100507-P10	Pack of 10 columns*
M100507-P30	Pack of 30 columns*
M100507-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Quencher-470

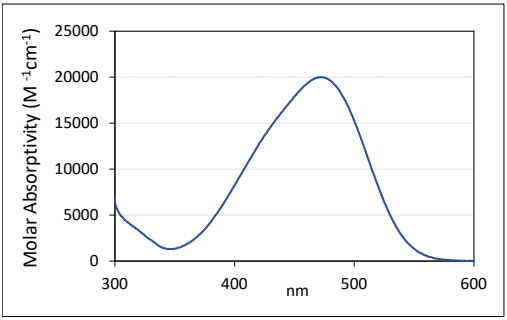
Acronym: Q-470



Quencher-470 is a Dabcyl-based non-fluorescent quencher. Its principal application is a quencher for blue and green fluorescent donor dyes for FRET probes. The quencher is fully compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum*	470 nm
Extinction coefficient (260 nm)	9,150 $M^{-1}cm^{-1}$
Extinction coefficient (470 nm)	22,800 $M^{-1}cm^{-1}$

**Measured for T_8-Q470 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

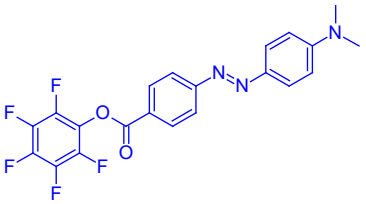


Available Q470 products	Product number
Quencher-470 PFP ester	M830572
Quencher-470 Internal Phosphoramidite	M830565
Quencher-470 CPG	M830562
Quencher-470 Polystyrene**	M830568 (bulk), M100446 (columns)

***Suitable for ABI 3900 DNA synthesizer*

Quencher-470 PFP ester

Product number: M830572; CAS #: 2378004-22-7



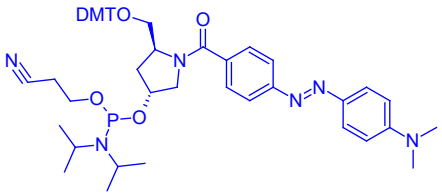
Formula	$C_{21}H_{14}F_5N_3O_2$
Molecular weight (reagent)	435.35 Da
Molecular weight (incorporated)	251.28 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830572	bulk
M830572-1MG	1 mg
M830572-10MG	10 mg

Quencher-470 Internal Phosphoramidite

Product number: M830565; CAS #: 2378004-32-9

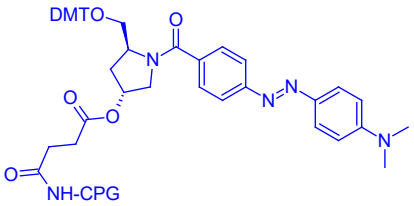


Formula	$C_{50}H_{59}N_6O_6P$
Molecular weight (reagent)	871.01 Da
Molecular weight (incorporated)	430.39 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling condition	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830565	bulk
M830565-50UMOL	50 µmol
M830565-100UMOL	100 µmol
M830565-250MG	250 mg
M830565-1000MG	1 g

Quencher-470 CPG

Product number: M830562

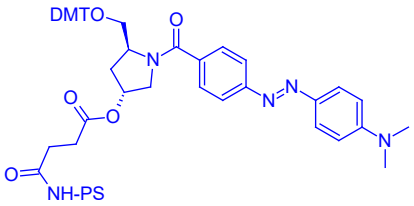


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	430.39 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830562	bulk
M830562-100MG	100 mg
M830562-250MG	250 mg
M830562-1000MG	1 g

Quencher-470 Polystyrene

Product numbers: M830568 (bulk), M100446 (columns)



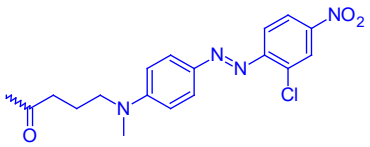
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	430.39 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Coupling efficiency test	N/A
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830568	bulk
M100446-P3	Pack of 3 columns*
M100446-P10	Pack of 10 columns*
M100446-P30	Pack of 30 columns*
M100446-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Eclipse Dark Quencher

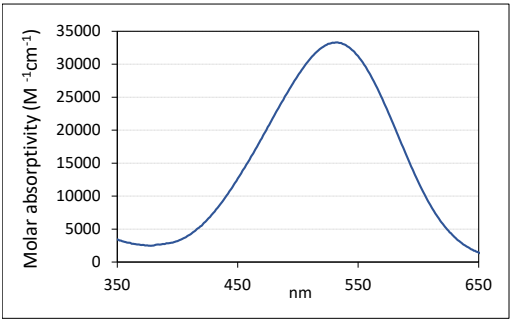
Synonyms: Eclipse Quencher, EDQ



Eclipse dark quencher is a universal non-fluorescent quencher for FRET probes. The quencher is fully compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum*	530 nm
Extinction coefficient (260 nm)	6,600 $M^{-1}cm^{-1}$
Extinction coefficient (530 nm)	33,300 $M^{-1}cm^{-1}$

**Measured for T_8-Eclipse Quencher conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

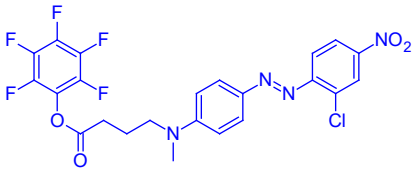


Available Eclipse Quencher products	Product number
Eclipse Dark Quencher PFP ester	M830573
Eclipse Dark Quencher Phosphoramidite	M830028
Eclipse Dark Quencher CPG	M830386
Eclipse Dark Quencher Polystyrene**	M830486 (bulk), M100447 (columns)
EDQ-Super I 3'-Phosphoramidite	M830781

***Suitable for ABI 3900 DNA synthesizer*

Eclipse Dark Quencher PFP ester

Product number: M830573; CAS #: 2378004-34-1



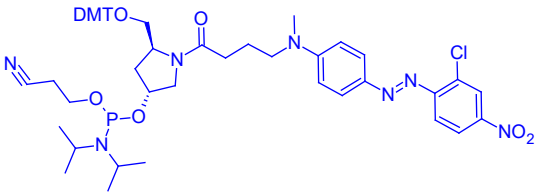
Formula	$C_{23}H_{16}ClF_5N_4O_4$
Molecular weight (reagent)	542.84 Da
Molecular weight (incorporated)	358.77 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830573	bulk
M830573-1MG	1 mg
M830573-10MG	10 mg

Eclipse Dark Quencher Phosphoramidite

Product number: M830028; CAS #: 2378004-24-9

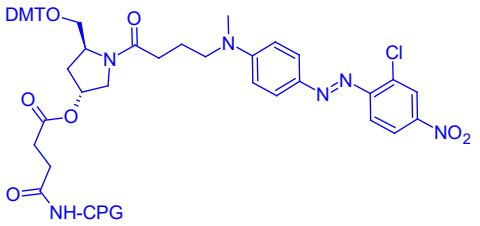


Formula	$C_{52}H_{61}ClN_7O_8P$
Molecular weight (reagent)	978.51 Da
Molecular weight (incorporated)	537.89 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830028	bulk
M830028-50UMOL	50 µmol
M830028-100UMOL	100 µmol
M830028-250MG	250 mg
M830028-1000MG	1 g

Eclipse Dark Quencher CPG

Product number: M830386

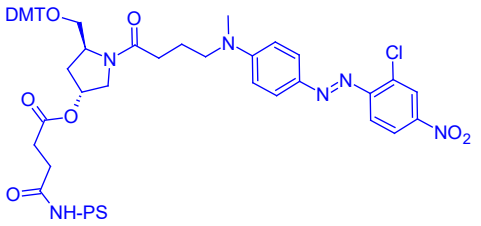


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	537.89 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830386	bulk
M830386-100MG	100 mg
M830386-250MG	250 mg
M830386-1000MG	1 g

Eclipse Dark Quencher Polystyrene

Product numbers: M830486 (bulk), M100447 (columns)



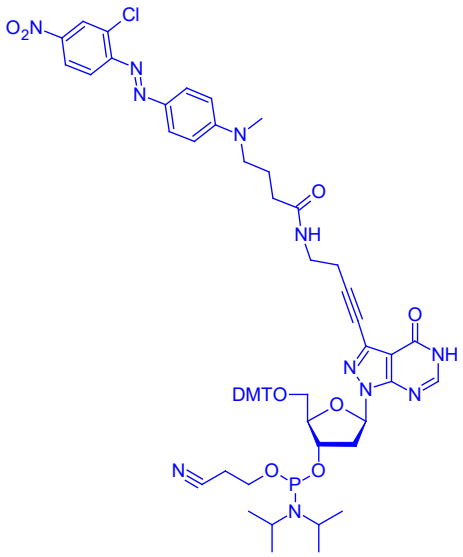
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	537.89 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830486	bulk
M100447-P3	Pack of 3 columns*
M100447-P10	Pack of 10 columns*
M100447-P30	Pack of 30 columns*
M100447-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

EDQ-Super I 3’-Phosphoramidite

Product number: M830781; CAS #: 2378004-12-5

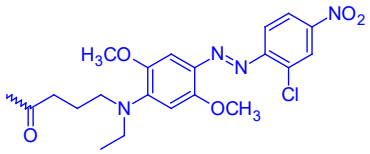


Formula	$C_{61}H_{67}ClN_{11}O_{10}P$
Molecular weight (reagent)	1180.68 Da
Molecular weight (incorporated)	740.05 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 3 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830781	bulk
M830781-50UMOL	50 µmol
M830781-100UMOL	100 µmol
M830781-250MG	250 mg
M830781-1000MG	1 g

Quencher-575

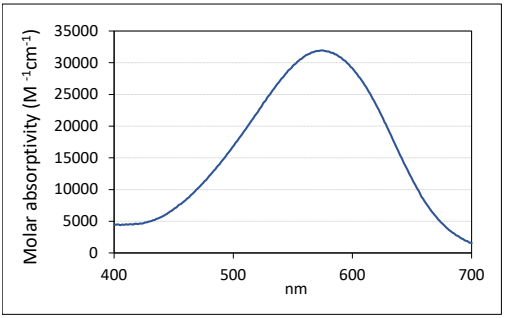
Acronym: Q-575



Quencher-575 quencher is a universal non-fluorescent quencher for FRET probes. The quencher is compatible with standard oligonucleotide synthesis. Mild deprotection is recommended.

Absorbance maximum*	575 nm
Extinction coefficient (260 nm)	4,200 $M^{-1}cm^{-1}$
Extinction coefficient (530 nm)	31,900 $M^{-1}cm^{-1}$

**Measured for T_8-Q575 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

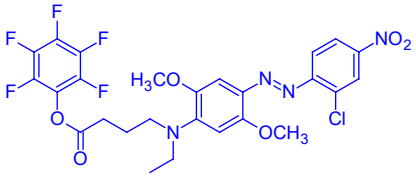


Available Q575 products	Product number
Quencher-575 PFP ester	M830574
Quencher-575 Internal Phosphoramidite	M830566
Quencher-575 CPG	M830563
Quencher-575 Polystyrene**	M830569 (bulk), M100448 (columns)

***Suitable for ABI 3900 DNA synthesizer*

Quencher-575 PFP ester

Product number: M830574; CAS #: 2378004-39-6



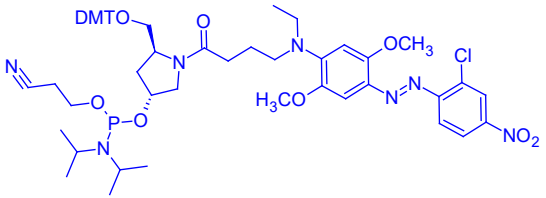
Formula	$C_{26}H_{22}ClF_5N_4O_6$
Molecular weight (reagent)	616.92 Da
Molecular weight (incorporated)	432.85 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830574	bulk
M830574-1MG	1 mg
M830574-10MG	10 mg

Quencher-575 Internal Phosphoramidite

Product number: M830566; CAS #: 2378004-36-3

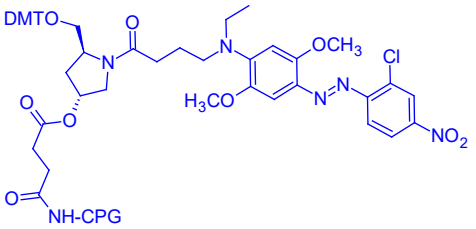


Formula	$C_{55}H_{67}ClN_7O_{10}P$
Molecular weight (reagent)	1052.59 Da
Molecular weight (incorporated)	611.96 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830566	bulk
M830566-50UMOL	50 µmol
M830566-100UMOL	100 µmol
M830566-250MG	250 mg
M830566-1000MG	1 g

Quencher-575 CPG

Product number: M830563

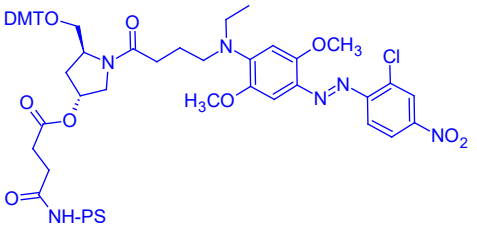


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	611.96 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥80%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830563	bulk
M830563-100MG	100 mg
M830563-250MG	250 mg
M830563-1000MG	1 g

Quencher-575 Polystyrene

Product numbers: M830569 (bulk), M100448 (columns)



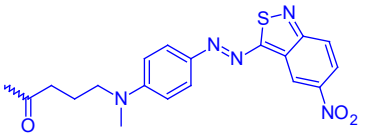
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	611.96 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥80%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 10 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830569	bulk
M100448-P3	Pack of 3 columns*
M100448-P10	Pack of 10 columns*
M100448-P30	Pack of 30 columns*
M100448-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Quencher-630

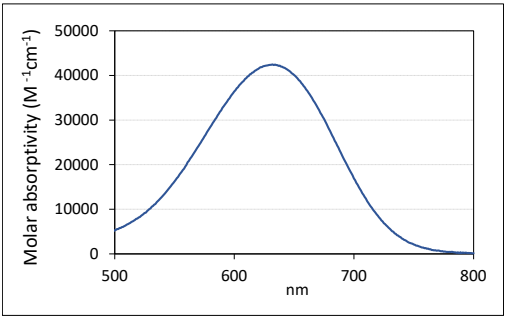
Acronym: Q-630



Quencher-630 is the most red-shifted quencher currently available from ELITechGroup, and it is especially suitable for quenching red and far red fluorophores including the AP680 dye. The quencher is compatible with standard oligonucleotide synthesis. Mild deprotection is recommended.

Absorbance maximum*	630 nm
Emission maximum*	N/A
Extinction coefficient (260 nm)	11,160 $M^{-1}cm^{-1}$
Extinction coefficient (630 nm)	42,400 $M^{-1}cm^{-1}$

**Measured for T_8-Q630 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*

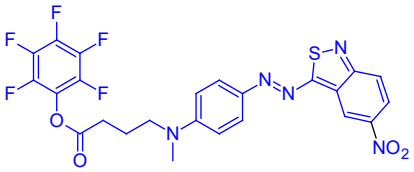


Available Q630 products	Product number
Quencher-630 PFP ester	M830575
Quencher-630 Internal Phosphoramidite	M830567
Quencher-630 CPG	M830564
Quencher-630 Polystyrene**	M830570 (bulk), M100449 (columns)

***Suitable for ABI 3900 DNA synthesizer*

Quencher-630 PFP ester

Product number: M830575; CAS #: 2378004-33-0



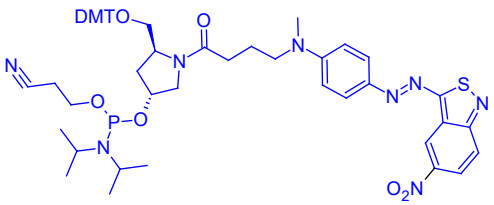
Formula	$C_{24}H_{16}F_5N_5O_4S$
Molecular weight (reagent)	565.47 Da
Molecular weight (incorporated)	381.40 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830575	bulk
M830575-1MG	1 mg
M830575-10MG	10 mg

Quencher-630 Internal Phosphoramidite

Product number: M830567; CAS #: 2378004-29-4

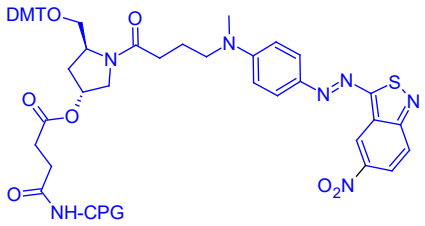


Formula	$C_{53}H_{61}N_8O_8PS$
Molecular weight (reagent)	1001.14 Da
Molecular weight (incorporated)	560.51 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥90%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	$^tBuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830567	bulk
M830567-50UMOL	50 µmol
M830567-100UMOL	100 µmol
M830567-250MG	250 mg
M830567-1000MG	1 g

Quencher-630 CPG

Product number: M830564

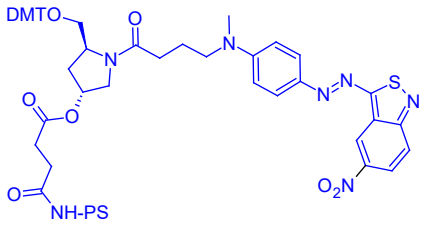


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	560.51 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830564	bulk
M830564-100MG	100 mg
M830564-250MG	250 mg
M830564-1000MG	1 g

Quencher-630 Polystyrene

Product numbers: M830570 (bulk), M100449 (columns)

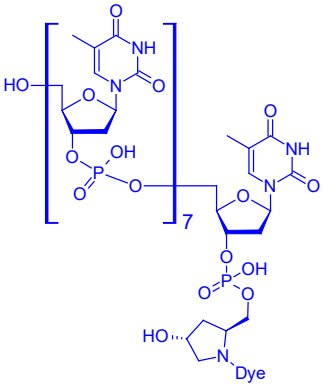


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	560.51 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830570	bulk
M100449-P3	Pack of 3 columns*
M100449-P10	Pack of 10 columns*
M100449-P30	Pack of 30 columns*
M100449-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

4. Dye Calibrators



Dye calibrators are available for evaluation of our fluorescent dyes' properties and instrument calibrations. The calibrators are T_8 conjugates with the dyes attached to the 3'-terminus via the hydroxyprolinol linker. All conjugates are carefully purified and formulated as 0.1 mM solution in 1x TE buffer.

Purity by Mass spectroscopy	≥90%
Identity by Mass spectroscopy	± 0.5% of calculated molecular weight (MW)
Recommended storage conditions	≤ -10°C, protect from light
Stability	3 years at ≤ -10°C
Available documents	Certificate of analysis

Product Number	Dye Calibrator	Abs max (nm)	Em max (nm)	ε_{260} ($M^{-1}cm^{-1}$)	$\varepsilon_{\lambda\ max}$ ($M^{-1}cm^{-1}$)	MW	Hydro-phobicity*
M300585	T_8-EB380-HEG	381	460	82,600	20,000	3191.5	22.0
M300586	T_8-FAM-HEG	496	517	98,700	80,400	3292.4	19.2
M302132	T_8-GG	497	521	18,500	93,200	3320.6	21.5
M300587	T_8-AP525	527	549	117,900	99,300	3233.2	18.6
M830730	T_8-YY	525	547	94,000	86,500	3088.9	25.6
M301430	T_8-AP559	559	581	93,700	86,500	3227.8	25.6
M830700	T_8-AP570	570	592	109,500	101,100	3303.9	29.4
M830731	T_8-RR	580	594	75,200	64,500	2817.9	17.9
M300392	T_8-AP593	593	613	85,080	115,240	3272.5	29.9
M301715	T_8-AP639	639	655	24,650	129,300	3453.1	37.5
M300589	T_8-AP642	641	653	85,100	283,200	3227.4	33.9
M830724	T_8-AP662	662	676	81,000	241,100	3251.5	35.0
M300753	T_8-AP680	682	704	81,500	221,300	3209.5	35.8
M302123	T_8-AP690	689	709	23,500	141,900	3358.1	30.5

All absorption and emission data were obtained in 50 mM Tris-HCl pH 8.5 at 20°C.
**Acetonitrile concentration (%) required to elute a dye calibrator from C18 column (see Appendix A for details).*

5. Nucleic Acid Base Analogues

In the field of molecular diagnostics, modified nucleobases are often utilized to modulate hybridization properties of natural nucleic acids (NAs). Short NA fragments are used as probes to identify specific NA targets and as primers to amplify these targets. Depending on the application, it may be necessary to adjust duplex stability and specificity. For example, degeneracy of the genetic code, target polymorphism, and a high rate of pathogen mutagenesis often impart strict requirements on assay design, necessitating very short probes and amplification primers. In this case, the hybridization strength of natural NA bases may not be sufficient to satisfy the assay requirements and, therefore, demand the use of some hybridization-enhancing techniques. For other applications, it may be imperative to ignore sequence variations (polymorphisms), which would require overcoming the fundamental specificity of the natural A:T and G:C base-pairs. ELITechGroup has developed a number of artificial NA base analogues to help fulfill those requirements.

Super G®

Super G (8-aza-7-deazaguanosine) demonstrates approximately the same base pairing specificity as natural G. However, unlike natural G, due to the absence of the N-7 nitrogen atom, it cannot form non-canonical base pairs. As a result, Super G effectively eliminates undesired secondary structures inherent to natural G-rich sequences and improves hybridization efficiency. The Super G analogue is recommended for use in NA probes and primers to disrupt G stretches.

An additional benefit of Super G for fluorogenic hybridization probes is its low fluorescence quenching tendency, which is an intrinsic and often undesired property of natural G. The resultant Super G-modified probes demonstrate higher fluorescence efficiency and, therefore, assay sensitivity.

Super A®

Super A (7-hydroxybutynyl-2-amino-8-aza-7-deazaadenosine) is a duplex-stabilizing nucleoside analogue. The Super A:T base pair is much more stable than the natural A:T; it even surpasses the 2-aminoadenosine:T base pair and approaches the stability of the G:C pair. Depending on length and sequence, each Super A incorporation will increase a probe or primer's

melting temperature (T_m) by 2.5-5.5°C. Generally, when Super A is located next to a G or C the stabilizing effect is larger (Ref. 14).

Super T®

Super T (5-hydroxybutynyl-2'-deoxyuridine) is another duplex-stabilizing nucleoside analogue which forms a stabilized Super T:A base pair with an average T_m increase of about 1.5-3°C per incorporation, presumably due to an increased stacking interaction between nucleotide bases (Ref. 12, 16). The stabilizing effect is highest when several Super Ts are incorporated consecutively.

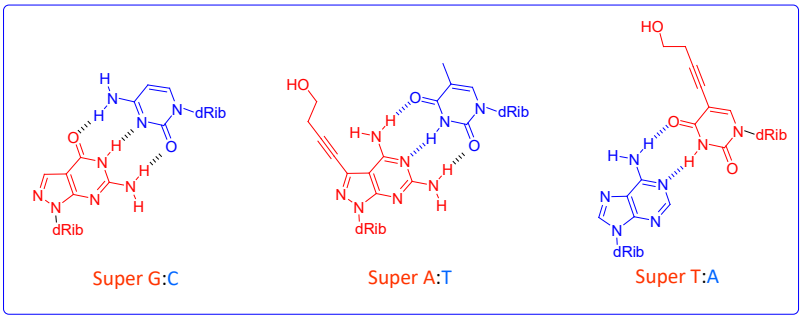


Figure 6 Structures of Super G, Super A, and Super T with their corresponding complementary base pairs.

Guidelines for incorporating Super A and Super T bases into PCR primers

An example of the typical benefits of primers containing modified bases is shown in Figure 7 where they are used to amplify the highly variable *gag* gene region of HIV. Only short sequences are conserved in that region and therefore available for primer design. The unmodified primer pair did not produce any visible PCR products on the agarose gel, whereas the primer pair stabilized by multiple incorporations of Super A and Super T (Forward ACCAAGGAAGC, Reverse CCTTCTGATAATGCTG) produced a good band of product with the expected 240 bp size. Other examples of successful Super A and Super T incorporation can be found in the research literature (Ref. 12, 13, 14, 15, 16, 17).

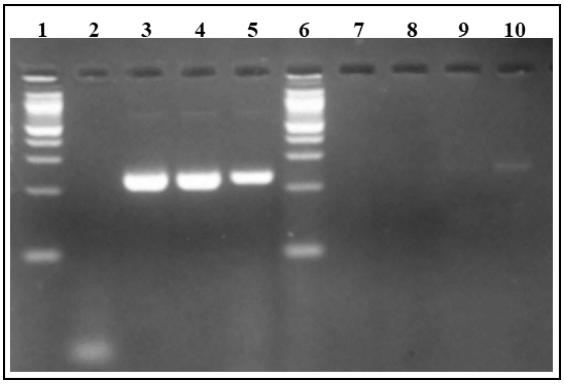


Figure 7 Stabilizing moieties of Super A and Super T in short primers improve amplification of highly variable templates.

Cloned HIV variants (gag gene region) were amplified with 0.5 µM each of modified or unmodified primers using JumpStart™ Taq ReadyMix™ (Sigma, St. Louis, MO, USA) using the following cycling parameters: 2 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. PCR products were run on a 4% agarose gel. Lanes 2-5 indicate the modified primer pair amplification and lanes 7-10 indicated the unmodified primer pair amplification. Lanes 2 &7 are no template controls. Lanes 3 & 8 contain the pN43 (wild type) plasmid template. Lanes 4 & 9 contain the pN43 – K103N (mutant) plasmid template. Lanes 5 & 10 contain the pN43 – V108I (mutant) plasmid template. Lanes 1 & 6 contain a 100 bp DNA ladder.

It should be noted that PCR efficiency is also affected by factors beyond the thermodynamic properties of primers. DNA polymerase must read through the modified bases without stuttering or pausing, and modified primers have to be efficiently extended. We have conducted experiments and summarized general guidelines for using Super A and Super T in PCR primers:

- Usual PCR T_m requirements should be used to ensure primer annealing.
- Neither Super A nor Super T should be used at the 3' end.
- Both Super A and Super T can be used as the second base from the 3' end.
- Super A should be used sparingly.
- Multiple Super A's should be separated by at least three natural bases or Super T.
- Super T can be used liberally.

Super I®

Super I™ is a nucleoside analogue that contains a quasi-universal nucleobase (7-aminobutynyl-8-aza-7-deazahypoxanthine) with improved hybridization and PCR enhancing properties (Ref. 18) as compared to commonly used 2'-deoxyinosine. It demonstrates improved ambiguity for pairing with A, T and C bases and its base pairing properties can be summarized as follows: X:C~X:A~X:T>X:G. The base pairing strength of Super I with A, T and C is approximately equal or slightly greater (depending on nearest neighbors) than the natural A:T but lower than the G:C, with the Super I:C base pair being slightly more stable than Super I:A and Super I:T. The improvement in PCR performance directly correlated with primers' T_m. Primers with multiple Super I incorporations can be used without significant inhibition of Taq polymerase activity provided the modifications are not used in stretches and are positioned more than 2 bases away from the 3' end.

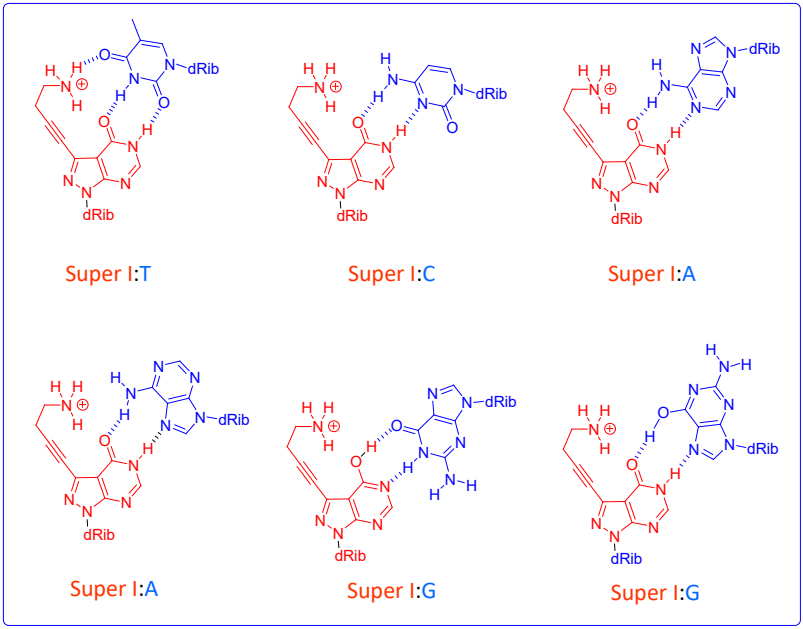
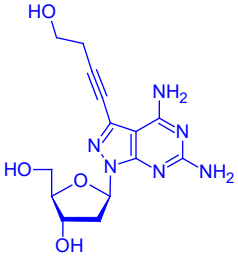


Figure 8 Possible hydrogen bonding patterns of Super I with natural nucleobases.

Comparison of Super I and 2'-deoxyinosine

We have determined that the stabilities of Super I:A, T and C base pairs are approximately equal or slightly greater (depending on nearest neighbors) than the natural A:T but lower than G:C, with Super I:C being slightly more stable than Super I:A and Super I:T. The beneficial stabilization effect of Super I pairs with A, T and C is especially pronounced for the multiply-substituted duplexes, which are generally more stable than the respective natural matched duplexes. In contrast, 2'-deoxyinosine forms base pairs with all four natural bases that are weaker than the A:T base pair. Moreover, multiple incorporations of 2'-deoxyinosine render modified duplexes much less stable than the respective natural matched duplexes. The much weaker Super I:G base pair is approximately equivalent to dI:G, both of which significantly destabilize the DNA duplexes. For practical applications such as PCR primer design where maintaining primer melting temperature is imperative, these results imply that single and especially multiple substitutions of A, T and G bases with Super I would be thermodynamically favorable whereas substitutions of cytosines should be avoided.

Super A®



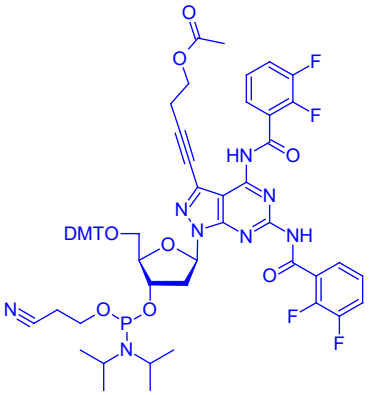
Super A (7-hydroxybutynyl-2-amino-8-aza-7-deazaadenosine) is a duplex-stabilizing nucleoside analogue. The Super A:T base pair is more stable than natural A:T and even surpasses the 2-aminoadenine:T base pair and approaches the stability of the G:C pair. Depending on length and sequence each Super A incorporation will increase the probe or primer's melting temperature (T_m) by 2.5-5.5°C.

Extinction coefficient (260 nm)	8,700 $M^{-1}cm^{-1}$

Available Super A products	**Product number**
Super A 3'-Phosphoramidite	M830249
Super A 5'-Phosphoramidite	M830250

Super A 3’-Phosphoramidite

Product number: M830249; CAS #: 2378004-08-9

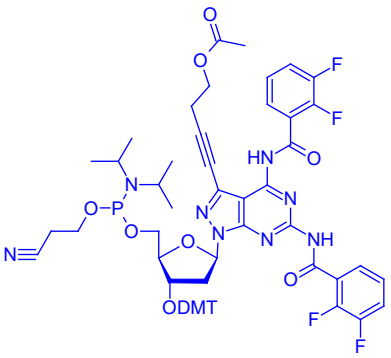


Formula	$C_{60}H_{59}F_4N_8O_{10}P$
Molecular weight (reagent)	1159.14 Da
Molecular weight (incorporated)	396.3 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16h @ 55°C or 2h @ 70°C. Extra deprotection time may be needed for multiple incorporations
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830249	bulk
M830249-50UMOL	50 µmol
M830249-100UMOL	100 µmol
M830249-250MG	250 mg
M830249-1000MG	1 g

Super A 5’-Phosphoramidite

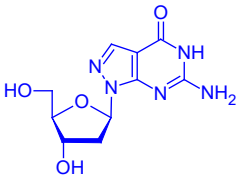
Product number: M830250 CAS #: 2378004-04-5



Formula	$C_{60}H_{59}F_4N_8O_{10}P$
Molecular weight (reagent)	1159.14 Da
Molecular weight (incorporated)	396.3 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16h @ 55°C or 2h @ 70°C. Extra time may be needed for multiple incorporations
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830250	bulk
M830250-50UMOL	50 µmol
M830250-100UMOL	100 µmol
M830250-250MG	250 mg
M830250-1000MG	1 g

Super G®



Super G (8-aza-7-deazaguanosine) eliminates undesired secondary structures inherent to natural G-rich sequences and provides improved hybridization efficiency. It is recommended for use in NA probes and primers to disrupt G stretches.

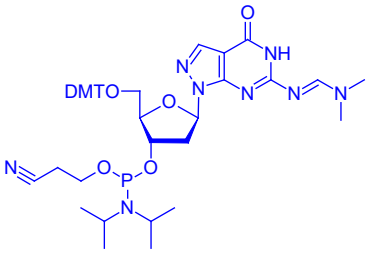
An additional benefit of Super G for use in fluorogenic hybridization probes is its low fluorescence quenching tendency, which is an intrinsic and often undesired property of natural G. The resultant Super G-modified probes demonstrate higher fluorescence efficiency and, therefore, assay sensitivity.

Extinction coefficient (260 nm)	12,400 $M^{-1}cm^{-1}$

Available Super G products	**Product number**
Super G 3'-Phosphoramidite	M830006
Super G 5'-Phosphoramidite	M830031

Super G 3'-Phosphoramidite

Product number: M830006; CAS #: 500891-26-9

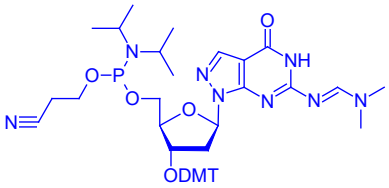


Formula	$C_{43}H_{53}N_8O_7P$
Molecular weight (reagent)	824.93 Da
Molecular weight (incorporated)	329.21 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830006	bulk
M830006-50UMOL	50 µmol
M830006-100UMOL	100 µmol
M830006-250MG	250 mg
M830006-1000MG	1 g

Super G 5’-Phosphoramidite

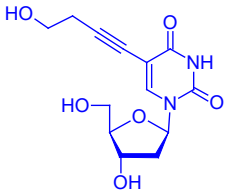
Product number: M830031; CAS #: 2378004-11-4



Formula	$C_{43}H_{53}N_8O_7P$
Molecular weight (reagent)	824.93 Da
Molecular weight (incorporated)	329.21 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 10 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830031	bulk
M830031-50UMOL	50 µmol
M830031-100UMOL	100 µmol
M830031-250MG	250 mg
M830031-1000MG	1 g

Super T®



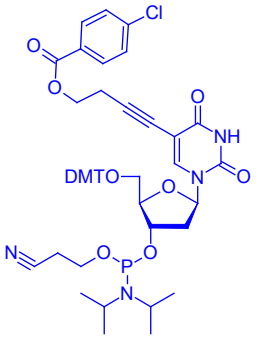
Super T (5-hydroxybutynyl-2'-deoxyuridine) is another duplex-stabilizing nucleoside analogue which forms a stabilized Super T:A base pair with an average T_m increase of about 2°C per incorporation. The Super T nucleobase can be used liberally in both probe and primer designs with little or no adverse effect on amplification efficiency.

Extinction coefficient (260 nm)	2,900 $M^{-1}cm^{-1}$

Available Super T products	**Product number**
Super T 3'-Phosphoramidite	M830040
Super T 5'-Phosphoramidite	M830041

Super T 3'-Phosphoramidite

Product number: M830040; CAS #: 2378004-13-6

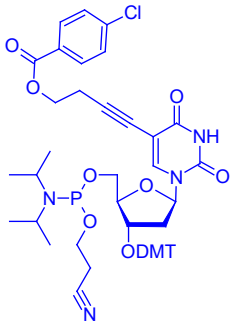


Formula	$C_{50}H_{54}ClN_4O_{10}P$
Molecular weight (reagent)	937.41 Da
Molecular weight (incorporated)	358.24 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 6 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830040	bulk
M830040-50UMOL	50 µmol
M830040-100UMOL	100 µmol
M830040-250MG	250 mg
M830040-1000MG	1 g

Super T 5’-Phosphoramidite

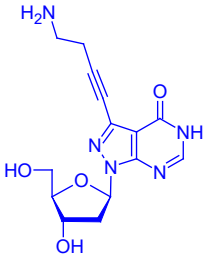
Product number: M830041; CAS #: 2378004-01-2



Formula	$C_{50}H_{54}ClN_4O_{10}P$
Molecular weight (reagent)	937.41 Da
Molecular weight (incorporated)	358.24 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 6 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830041	bulk
M830041-50UMOL	50 µmol
M830041-100UMOL	100 µmol
M830041-250MG	250 mg
M830041-1000MG	1 g

Super I®



Super I is a nucleoside analogue that contains a quasi-universal nucleobase (7-aminobutynyl-8-aza-7-deazahypoxanthine) with improved hybridization and polymerase chain reaction (PCR) enhancing properties as compared to commonly used 2'-deoxyinosine.

It demonstrates improved ambiguity for pairing with A, T and C bases and its base pairing properties can be summarized as follows: X:C~X:A~X:T>X:G. The base pairing strength of Super I with A, T and C is approximately equal or slightly greater (depending on nearest neighbors) than natural A:T but lower than G:C, with the Super I:C base pair being slightly more stable than Super I:A and Super I:T. The improvement in PCR performance directly correlated with primers' T_m.

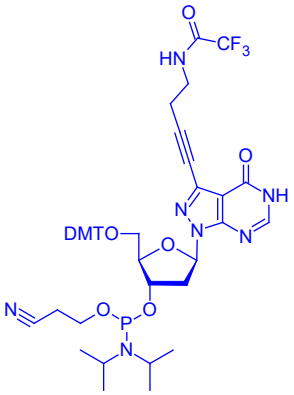
Primers with multiple Super I incorporations can be used without noticeable inhibition of Taq polymerase activity provided the modifications are not used in stretches and are positioned more than 2 bases away from the 3' end.

Extinction coefficient (260 nm)	10,000 $M^{-1}cm^{-1}$

Available Super I products	**Product number**
Super I 3'-Phosphoramidite	M830193
Super I 5'-Phosphoramidite	M830712

Super I 3’-Phosphoramidite

Product number: M830193; CAS #: 1401110-57-3

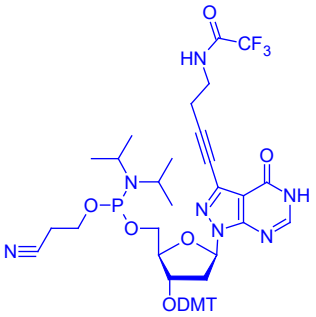


Formula	$C_{46}H_{51}F_3N_7O_8P$
Molecular weight (reagent)	917.91 Da
Molecular weight (incorporated)	381.28 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N’,N’-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 8 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830193	bulk
M830193-50UMOL	50 µmol
M830193-100UMOL	100 µmol
M830193-250MG	250 mg
M830193-1000MG	1 g

Super I 5’-Phosphoramidite

Product number: M830712; CAS #: 2378004-03-4



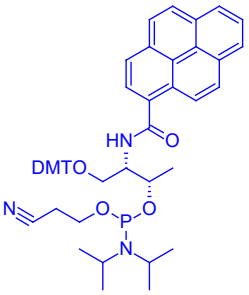
Formula	$C_{46}H_{51}F_3N_7O_8P$
Molecular weight (reagent)	917.91 Da
Molecular weight (incorporated)	381.28 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N’,N’-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 8 years
Available documents	Certificate of analysis, SDS

Catalog number	Unit size
M830712	bulk
M830712-50UMOL	50 µmol
M830712-100UMOL	100 µmol
M830712-250MG	250 mg
M830712-1000MG	1 g

6. Duplex Intercalators

Pyrene Internal Phosphoramidite

Product number: M830711; CAS #: 2378724-62-8



Pyrene-functionalized oligonucleotides are useful tools in fundamental research, diagnostics, and materials science. Their popularity is linked to the ability of pyrenes to function as polarity-sensitive and quenchable fluorophores, excimer-generating units, aromatic stacking moieties, and nucleic acid duplex intercalators (Ref. 28). Pyrene demonstrates a long excited state lifetime, a large Stokes shift, and chemical stability.

Pyrene internal phosphoramidite (M830711) is prepared by coupling of 1-pyrenecarboxylic acid to D-threoninol, a trifunctional linker with an amino group and two hydroxyl groups in a specific steric bond configuration. The pyrene moiety in this combination is a universal base capable of binding equally well with all four natural nucleobases (unpublished data). To achieve optimal backbone configuration the phosphoramidite should be used in conjunction with 5'-nucleoside phosphoramidites for probe synthesis.

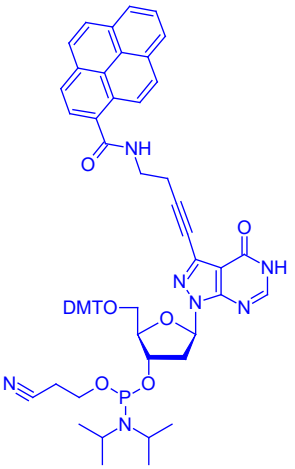
The phosphoramidite is suitable for internal as well as terminal incorporation and compatible with standard DNA synthesis and both standard and mild deprotection conditions.

Formula	$C_{51}H_{54}N_3O_6P$
Molecular weight (reagent)	835.96 Da
Molecular weight (incorporated)	395.34 Da
Extinction coefficient (260 nm)	12,000 $M^{-1}cm^{-1}$
Purity (reverse phase HPLC)	$\geq$80%
Purity of test oligo (reverse phase HPLC)	$\geq$80%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	$\leq$ -10°C, dry/ 7 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830711	bulk
M830711-50UMOL	50 µmol
M830711-100UMOL	100 µmol
M830711-250MG	250 mg
M830711-1000MG	1 g

Pyrene-Super I 3'-Phosphoramidite

Product number: M830782; CAS #: 2378004-05-6

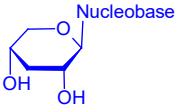


Formula	$C_{61}H_{60}N_7O_8P$
Molecular weight (reagent)	1050.14 Da
Molecular weight (incorporated)	609.52 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830782	bulk
M830782-50UMOL	50 µmol
M830782-100UMOL	100 µmol
M830782-250MG	250 mg
M830782-1000MG	1 g

7. 3'-Deoxyribopyranosyl (4'→2') Nucleic Acid

Acronym: p-DNA



3'-Deoxy-β-D-ribopyranosyl nucleic acids (p-DNA) are polymers that preferentially pair with p-DNA versus natural DNA sequences (Ref. 29). p-DNAs form antiparallel, exclusively Watson-Crick-paired duplexes that are much stronger than corresponding DNA duplexes.

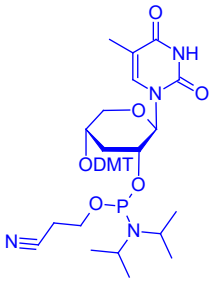
For some applications, it is essential to be able to design large numbers of nucleic acid sequences with the same T_m that hybridize efficiently and specifically with respective targets while not cross-hybridizing with each other (Ref. 30). These so-called T_m-leveled orthogonal nucleic acids can be designed using p-DNA monomers (Ref. 31). Orthogonal p-DNAs with high duplex stability, low affinity for DNA, and which are not recognized by DNA processing enzymes are useful for labeling, barcoding, or anchoring multiple DNA-containing substrates co-existing in one mixture, on one array, or on a lateral flow strip.

p-DNA monomers are available as 4'-DMT, 2'-phosphoramidites and are fully compatible with standard DNA synthesis. In particular, synthesis of chimeric DNA – p-DNA can be achieved via standard synthesis.

Available p-DNA products	Product number
p-DNA T Phosphoramidite	M830779
p-DNA A Phosphoramidite	M830776
p-DNA C Phosphoramidite	M830777
p-DNA G Phosphoramidite	M830778

p-DNA T Phosphoramidite

Product number: M830779; CAS #: 256648-34-7

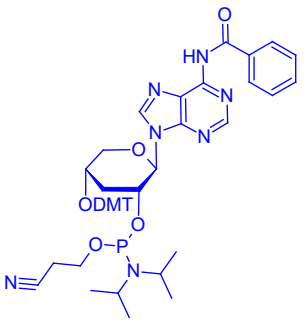


Formula	$C_{40}H_{49}N_4O_8P$
Molecular weight (reagent)	744.81 Da
Molecular weight (incorporated)	304.19 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830779	bulk
M830779-50UMOL	50 µmol
M830779-100UMOL	100 µmol
M830779-250MG	250 mg
M830779-1000MG	1 g

p-DNA A Phosphoramidite

Product number: M830776; CAS #: 256648-33-6

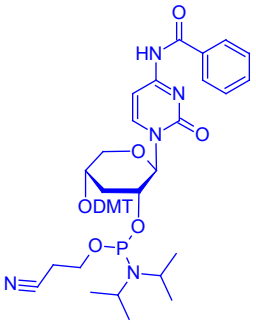


Formula	$C_{47}H_{52}N_7O_7P$
Molecular weight (reagent)	857.93 Da
Molecular weight (incorporated)	313.20 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830776	bulk
M830776-50UMOL	50 µmol
M830776-100UMOL	100 µmol
M830776-250MG	250 mg
M830776-1000MG	1 g

p-DNA C Phosphoramidite

Product number: M830777; CAS #: 2378004-09-0

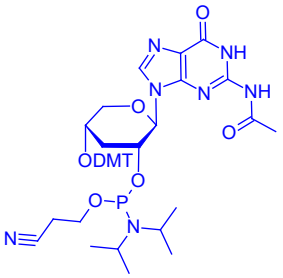


Formula	$C_{46}H_{52}N_5O_8P$
Molecular weight (reagent)	833.91 Da
Molecular weight (incorporated)	289.18 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830777	bulk
M830777-50UMOL	50 µmol
M830777-100UMOL	100 µmol
M830777-250MG	250 mg
M830777-1000MG	1 g

p-DNA G Phosphoramidite

Product number: M830778; CAS #: 457880-65-8



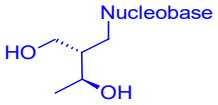
Formula	$C_{42}H_{50}N_7O_8P$
Molecular weight (reagent)	811.86 Da
Molecular weight (incorporated)	329.20 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830778	bulk
M830778-50UMOL	50 µmol
M830778-100UMOL	100 µmol
M830778-250MG	250 mg
M830778-1000MG	1 g

8. Non-constrained Nucleic Acid

Acronym: NNA

Non-constrained Nucleic Acid (NNA) is an artificial acyclic phosphodiester DNA analogue composed of stereoisomerically pure (S,S)-2-(nucleobase-methyl)butane-1,3-diols.



The (S,S)-stereoisomer of NNA forms homoduplexes of greater stability than DNA oligonucleotides of the same sequences and does not hybridize with natural DNA to form heteroduplexes (Ref. 32). For a 10-12-mer, on average, the NNA homoduplexes' melting temperature (T_m) is 8°C higher than the respective DNA (Figure 9) and about 8°C lower than the p-DNA duplex depending on nucleobase composition.

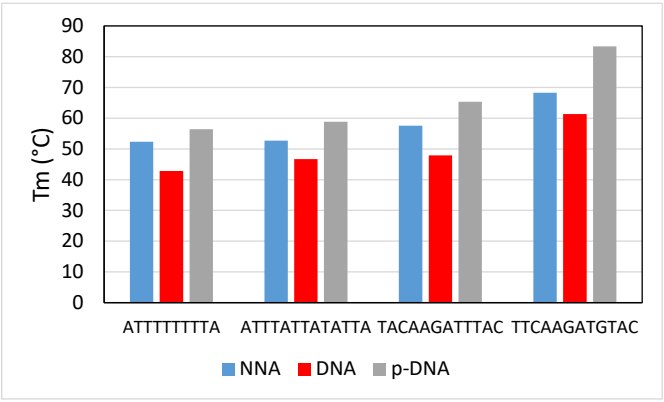


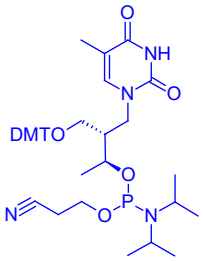
Figure 9 Melting temperature (T_m) comparison of NNA, DNA and p-DNA duplexes of different nucleobase composition.

NNA, as a tuned down alternative to p-DNA, is also not recognizable by DNA processing enzymes and is useful for labeling, barcoding or anchoring of multiple DNA-containing substrates co-existing in one mixture, array, or lateral flow strip.

Available NNA products	Product number
NNA T Phosphoramidite	M830775
NNA A Phosphoramidite	M830772
NNA C Phosphoramidite	M830773
NNA G Phosphoramidite	M830774

NNA T Phosphoramidite

Product number: M830775; CAS #: 2378004-25-0

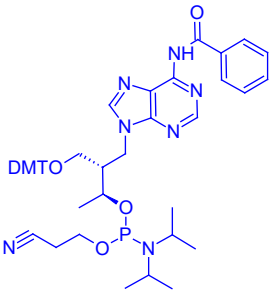


Formula	$C_{40}H_{51}N_4O_7P$
Molecular weight (reagent)	730.83 Da
Molecular weight (incorporated)	290.20 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830775	bulk
M830775-50UMOL	50 µmol
M830775-100UMOL	100 µmol
M830775-250MG	250 mg
M830775-1000MG	1 g

NNA A Phosphoramidite

Product number: M830772; CAS #: 2378004-35-2

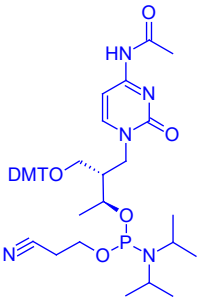


Formula	$C_{47}H_{54}N_7O_6P$
Molecular weight (reagent)	843.95 Da
Molecular weight (incorporated)	299.22 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830772	bulk
M830772-50UMOL	50 µmol
M830772-100UMOL	100 µmol
M830772-250MG	250 mg
M830772-1000MG	1 g

NNA C Phosphoramidite

Product number: M830773; CAS #: 2378004-44-3

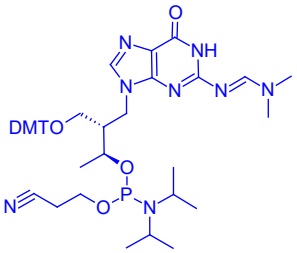


Formula	$C_{41}H_{52}N_5O_7P$
Molecular weight (reagent)	757.85 Da
Molecular weight (incorporated)	275.19 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830773	bulk
M830773-50UMOL	50 µmol
M830773-100UMOL	100 µmol
M830773-250MG	250 mg
M830773-1000MG	1 g

NNA G Phosphoramidite

Product number: M830774; CAS #: 2378004-41-0



Formula	$C_{43}H_{55}N_8O_6P$
Molecular weight (reagent)	810.92 Da
Molecular weight (incorporated)	315.22 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^{1}H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Multiple phosphoramidite additions and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830774	bulk
M830774-50UMOL	50 µmol
M830774-100UMOL	100 µmol
M830774-250MG	250 mg
M830774-1000MG	1 g

9. $CDPI_3$ MGB™-Oligonucleotide conjugates and their applications

The tripeptide of dihydropyrroloindole-carboxylate ($CDPI_3$) (Ref. 19) (Figure 10) is a minor groove binding (MGB) moiety derived from the natural product CC-1065 with strong DNA binding properties. $CDPI_3$ MGB is a crescent-shaped molecule which binds isohelically within the B-form DNA minor groove.

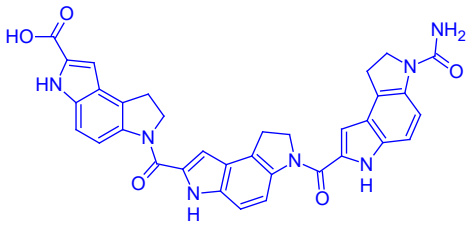


Figure 10 1,2-Dihydro-(*3H*)-pyrrolo[3,2-e]indole-7-carboxylate tripeptide ($CDPI_3$).

The reversible binding is mediated via hydrophobic and van der Waals interactions between the MGB and the floor of the groove. The MGB moiety occupies a region of duplex DNA approximately 5 bases long (Figure 11) and binds to both A/T and G/C rich sequences with association constants of K_a ~$1x10^7$ M^{-1} and K_a~$1x10^5$ M^{-1}, respectively.

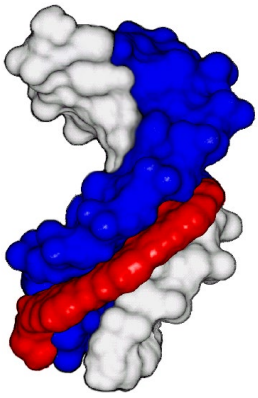


Figure 11 Connolly surface representation of a decamer DNA duplex formed between an MGB-ODN and its complement (Ref. 20). Red represents the MGB and linker moieties, blue is the MGB-labeled strand and light gray is the complement strand.

Synthetic oligonucleotides (ODNs) with covalently attached $CDPI_3$ MGB moieties were first introduced in 1995 by Lukhtanov et al. (Ref. 21). It has since been shown that such ODNs have enhanced DNA affinity and have improved the hybridization properties of sequence-specific DNA probes. Short MGB-oligonucleotides hybridize with single-stranded DNA to give more stable DNA duplexes than unmodified ODNs of similar length. For example, a DNA duplex formed between the $CDPI_3$ MGB-$(dT)_8$ conjugate and poly(dA) template has a melting temperature (T_m) that is 44°C higher than an unmodified duplex. Mismatch discrimination of short MGB-ODNs is also enhanced versus unmodified oligonucleotides. It was demonstrated that mismatches under the MGB binding region were more easily discriminated for a 15-mer MGB-ODN versus an unmodified ODN with up to a 3-fold increase in free energy difference (Ref. 22).

The tethered $CDPI_3$ MGB moiety has a preference for an A/T rich, B-form DNA duplex but is capable of binding to DNA duplexes with mixed sequences as well as to some modified backbone nucleic acids, as investigated by Kutyavin et al. (Ref. 23). The preference for A/T sequences has a useful practical application—it is well known that DNA duplex T_m depends on A/T and G/C content with A/T rich sequences being much less stable. $CDPI_3$ MGB tethering significantly reduces the difference, such that probes of equal length have similar T_m values regardless of base composition.

Applications

1. Arrest of primer extension and PCR blockers

$CDPI_3$ MGB-ODN conjugates were investigated for potential use as antigene agents via the inhibition of DNA polymerase (Ref. 24). The study, which was done in the context of a single-stranded DNA phage, demonstrated that T7 DNA polymerase was physically blocked when a complementary 16-mer 5'-$CDPI_3$ MGB-ODN was hybridized to a downstream site. Blockage was abolished when a single mismatch was introduced. A 16-mer with 3'-$CDPI_3$ MGB moiety also failed to arrest primer extension. The exceptional efficiency of the primer extension arrest was attributed to DNA polymerase's inability to displace the 5' end of the duplex super-stabilized with the $CDPI_3$ MGB group.

It has since been shown that 5'-$CDPI_3$ MGB-ODNs are able to arrest Taq DNA polymerase and therefore can be used at PCR temperatures as well. This came as a surprise since it was expected that the 5' exonuclease Taq polymerase activity would degrade such duplexes. Evidently, 5'-MGB labeling makes ODNs resistant to 5' exonuclease digestion. Such ODNs can

be used as PCR blockers to prevent amplification of selected DNA sequences.

2. Short and fluorogenic PCR primers

Efficient priming of PCR was demonstrated with 5'-MGB-ODNs as short as 8-mers using modified (touch-down) PCR cycling conditions or 10-16-mers using a regular PCR cycle (Ref. 25). The PCR was shown to produce specific amplification products of expected size. The reduced-length primers were suggested for use for PCR amplification of viral sequences which possess a high degree of variability or techniques such as gene hunting and differential display which amplify multiple sequences using short primer pairs.

5'-MGB-primers which also have an attached 5'-fluorophore are able to quench the dye fluorescence via the photo-induced electron transfer (PET) mechanism, depicted in Figure 12. Such primers are significantly quenched in a single strand state but become highly fluorescent when incorporated into the PCR amplicon allowing for detection of target amplification.

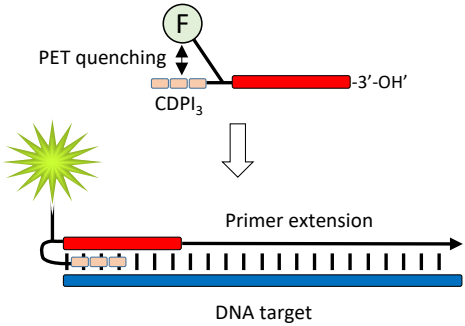


Figure 12 Mechanism of action of fluorogenic $CDPI_3$ MGB-Fluorophore primers.

3. Real-time PCR probes

The stronger binding of MGB-ODNs versus unmodified ODNs allows for more stringent hybridization conditions to be used in DNA hybridization assays. Short MGB-ODNs with improved mismatch discrimination are especially useful in PCR assays since they bind efficiently and specifically during the high-temperature primer extension cycle. Several types of real-time PCR probes that utilize the MGB moiety have been developed.

- MGB TaqMan® probes (Ref. 22) have $CDPI_3$ MGB-Quencher and Fluorophore tethered to the 3' and 5' ends, respectively. Provided that specific sequence is present in the target DNA, the TaqMan

probes are degraded by Taq polymerase during PCR releasing unquenched fluorophore.

- MGB Eclipse® hybridization probes (Ref. 13) have the $CDPI_3$ MGB-Quencher and Fluorophore attached at the 5' and 3' ends, respectively. They are non-degradable and their fluorescence is strongly increased upon the probes' hybridization to amplified targets during the annealing step.
- MGB Pleiades® probes (Ref. 8) are also non-degradable hybridization probes. They have the $CDPI_3$ MGB-Fluorophore and Quencher moieties tethered to the 5' and 3' ends, respectively. These probes utilize the unique dual fluorescence quenching mechanism to significantly reduce background fluorescence and improve signal-to-background ratio.
- MGB FRET probes are similar to MGB Pleiades but contain an additional (donor) fluorophore conjugated to an internal nucleobase. MGB FRET probes allow for multicolor detection with a single wavelength excitation. MGB FRET probes offer high signal-to-background ratios due to low background fluorescence via the dual quenching mechanism.

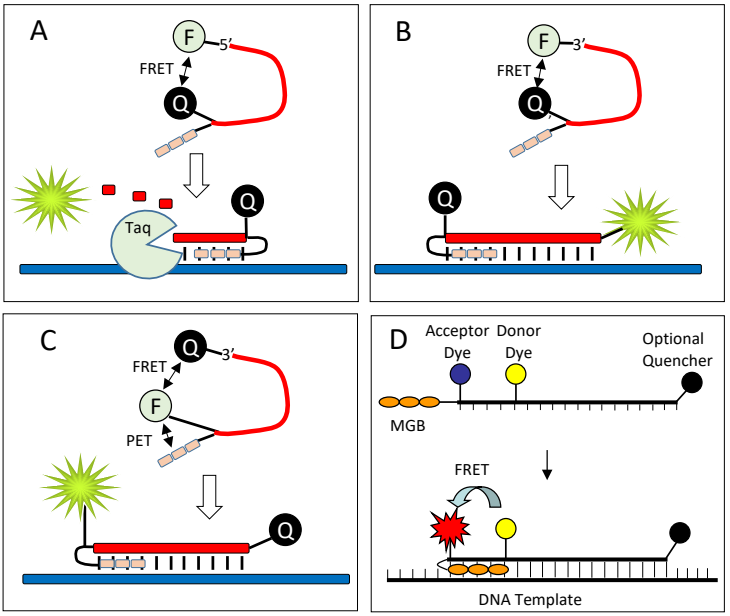


Figure 13 Putative mechanisms of action of MGB TaqMan (A), MGB Eclipse (B), MGB Pleiades (C) and MGB FRET (D) probes.

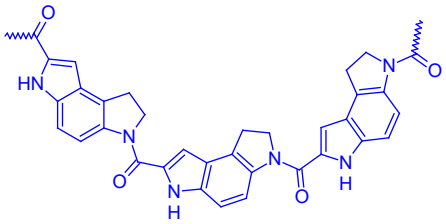
4. miRNA Inhibitors

In a recent US patent (Ref. 26), it was disclosed that MGB-ODNs with a 2'-OMe sugar-phosphate nucleic acid backbone are highly efficient and specific miRNA inhibitors. The inhibition is more pronounced when the minor groove binder is tethered to the 5'end of a miRNA inhibitor. The mechanism of this effect is not fully understood but could be attributed to the ability of 5'-MGB moiety to stabilize nucleic acid duplex and block enzymatic activities.

MGB-ODN-based miRNA inhibitors have also shown improved cellular uptake and promise as pharmaceuticals by modulating gene expression (Ref. 27).

$CDPI_3$ Minor Groove Binder

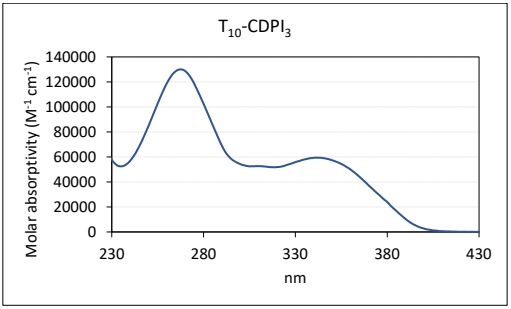
Acronym: $CDPI_3$ MGB



$CDPI_3$ MGB is an efficient duplex-stabilizing agent. This ligand improves background fluorescence and mismatch discrimination abilities of DNA probes. When attached to the 5'-end of a probe it inhibits the 5' → 3' exonuclease activity of DNA polymerases.

Absorbance maximum*	340 nm
Emission maximum*	N/A
Extinction coefficient (260 nm)	37,900 $M^{-1}cm^{-1}$**
Extinction coefficient (340 nm)	59,300 $M^{-1}cm^{-1}$

**Measured for T_{10}-$CDPI_3$ conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***$CDPI_3$ contribution only*

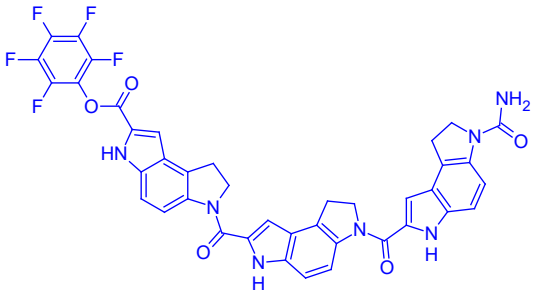


Available $CDPI_3$ MGB products	Product number
$CDPI_3$ MGB Terminal Phosphoramidite	M830252
$CDPI_3$ MGB CPG	M830739
$CDPI_3$ MGB Polystyrene***	M830203 (bulk), M100103 (columns)

****Suitable for ABI 3900 DNA synthesizer*

$CDPI_3$ PFP ester

Product number: M830187



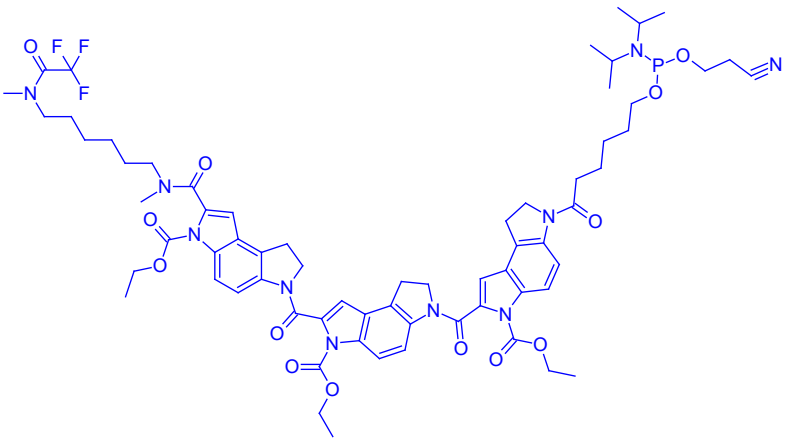
Formula	$C_{40}H_{26}F_5N_7O_5$
Molecular weight (reagent)	779.67 Da
Molecular weight (incorporated)	595.60 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830187	bulk
M830187-1MG	1 mg
M830187-10MG	10 mg

$CDPI_3$ MGB Terminal Phosphoramidite

Product number: M830252

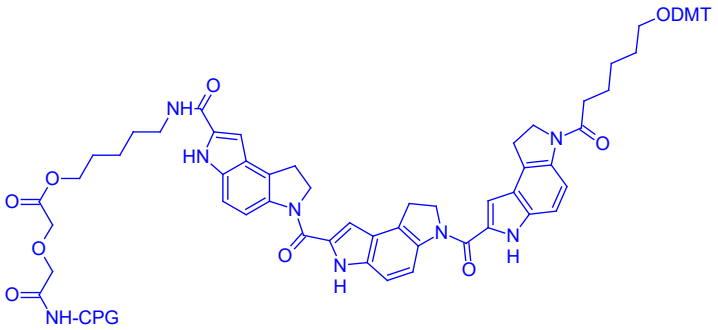


Formula	$C_{67}H_{82}F_3N_{10}O_{13}P$
Molecular weight (reagent)	1323.39 Da
Molecular weight (incorporated)	872.94 Da
Purity (reverse phase HPLC)	≥85%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity before/after coupling	^1H, ^{31}P NMR /Mass spectroscopy
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830252	bulk
M830252-50UMOL	50 µmol
M830252-100UMOL	100 µmol
M830252-250MG	250 mg
M830252-1000MG	1 g

CDPI$_3$ MGB CPG

Product number: M830739

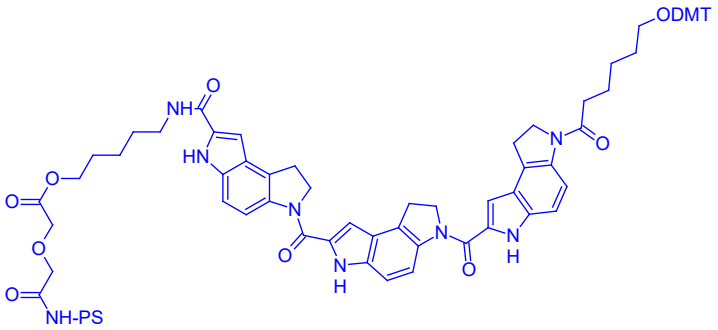


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	831.87 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 2 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830739	bulk
M830739-100MG	100 mg
M830739-250MG	250 mg
M830739-1000MG	1 g

CDPI$_3$ MGB Polystyrene

Product numbers: M830203 (bulk), M100103 (columns)



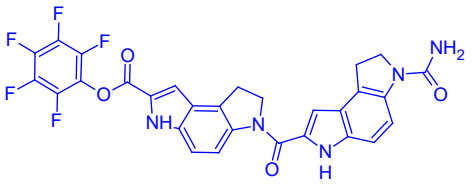
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	831.87 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830203	bulk
M100103-P3	Pack of 3 columns*
M100103-P10	Pack of 10 columns*
M100103-P30	Pack of 30 columns*
M100103-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

CDPI$_2$ PFP ester

Product numbers: M830790



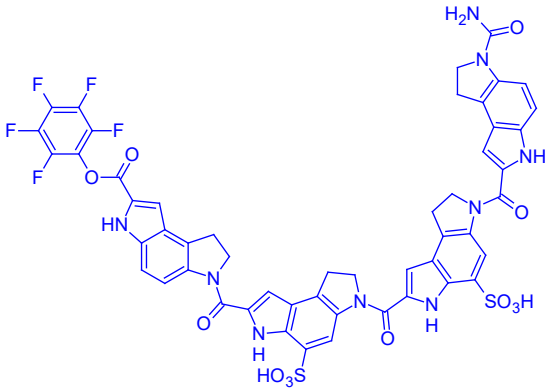
Formula	$C_{29}H_{18}F_5N_5O_4$
Molecular weight (reagent)	595.48 Da
Molecular weight (incorporated)	411.41 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830790	bulk
M830790-1MG	1 mg
M830790-10MG	10 mg

CDPI$_4$ (SO$_3$H)$_2$ PFP ester

Product numbers: M830188



Formula	$C_{69}H_{66}F_5N_{13}O_{12}S_2$ (bis-DBU salt)
Molecular weight (reagent)	1428.47 Da (bis-DBU salt)
Molecular weight (incorporated)	939.92 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830188	bulk
M830188-1MG	1 mg
M830188-10MG	10 mg

10. PBI_3 MGB™-Oligonucleotide conjugates

Piperazino-tris-benzimidazole (PBI_3) (Figure 14) is a DNA minor groove binder derived from the anthelmintic compound Hoechst 33258. It occupies a region of 5 to 6 base pairs in a DNA duplex and preferentially binds to A/T rich sequences.

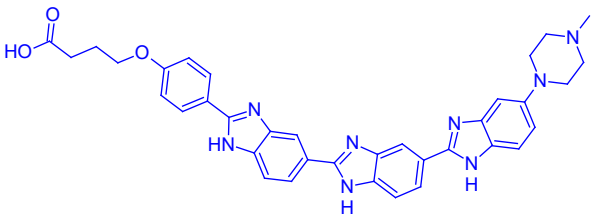


Figure 14 Structure of PBI_3 minor groove binder

Similar to $CDPI_3$, oligonucleotides with a conjugated PBI_3 moiety demonstrate enhanced hybridization properties (table below). While both types of MGB prefer A/T rich sequences, the degree of stabilization of such duplexes by PBI_3 is substantially higher, on average by 10°C for a 12-mer sequence with an A/T rich 5-6 base MGB binding region.

Comparison of DNA duplex stabilization (duplex T_m (°C)) by conjugated $CDPI_3$ and PBI_3 minor groove binders

5'-3'	5'-MGB*			PBI_3-$CDPI_3$
	None	$CDPI_3$	PBI_3	ΔT_m
TAAAAGGTGTAC	38.5	53.9	63.2	9.3
TATAAGGTGTAC	38.2	51.7	56.8	5.1
TATTTGGTGTAC	40.8	58.0	67.3	9.2
TAAAAATTGTAC	34.3	52.2	66.4	14.3
TAAAAAGTGTAC	34.7	52.8	63.2	10.4
ATTTTTTTGTAC	33.6	55.8	66.2	10.4
TTTTTTGTGTAC	34.9	58.6	67.9	9.3
TTTTTGGTGTAC	37.6	59.3	68.5	9.2
TACAAGGTGTAC	41.9	48.8	53.1	4.4
TGCAAGGTGTAC	46.8	52.2	54.8	2.6
TGCACGGTGTAC	51.7	56.2	56.9	0.6
TGCACCGTGTAC	52.1	56.4	57.3	0.9
Average T_m:	**40.1**	**55.1**	**62.4**	

**Also contained FAM-HEG (M830100) between MGB and 5'-end.*
Highlighted are probable MGB binding regions.

Stabilization of G/C rich sequences is comparably modest for both MGB types, 1-5°C for a 12-mer sequence.

Another aspect of an MGB conjugate is the ability to distinguish between fully (matched) and partially (mismatched) complementary duplexes. With regards to match versus mismatch, both $CDPI_3$ and PBI_3 demonstrate similar performance. On average, about 11°C duplex destabilization (T_m decrease) is observed in a 12-mer sequence when a single mismatched base pair is introduced.

Comparison of mismatch discrimination by conjugated $CDPI_3$ and PBI_3 minor groove binders

	Match-Mismatch (ΔT_m)			
5'-3'	**$CDPI_3$**	**PBI_3**	**Mismatch**	**Position**
TAAAAGGTGTAC	12.9	15.0	A/A	3
TAAAAGGTGTAC	11.4	11.5	G/T	6
TATAAGGTGTAC	10.3	7.4	T/T	3
TATTTGGTGTAC	9.2	12.9	A/A	2
TAAAAATTGTAC	27.9	31.1	A/C, T/C	6,7
TAAAAAGTGTAC	11.8	12.9	A/C	6
TAAAAAGTGTAC	10.8	9.8	G/A	7
ATTTTTTTGTAC	10.2	8.4	A/A, T/C	1,7
TTTTTTGTGTAC	14.7	12.9	T/C	6
TTTTTTGTGTAC	18.7	14.8	T/T, G/A	1,7
TTTTTGGTGTAC	12.9	13.0	T/T	2
TTTTTGGTGTAC	11.2	10.2	G/A	6
TACAAGGTGTAC	8.9	8.8	A/C	2
TGCAAGGTGTAC	10.0	10.0	G/T	2
TGCACGGTGTAC	17.5	14.5	C/T	5
TGCACGGTGTAC	12.7	13.0	G/G	6
TGCACCGTGTAC	15.7	17.4	C/C	6
Average ΔT_m:	**11.3**	**11.2**		

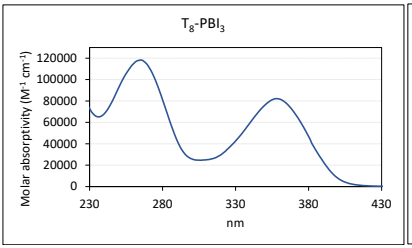
Despite the similarities, PBI_3 MGB is an attractive alternative to $CDPI_3$ when duplex hyper-stabilization is required, for instance, for a sequence specific polymerase arrest (PCR blocking).

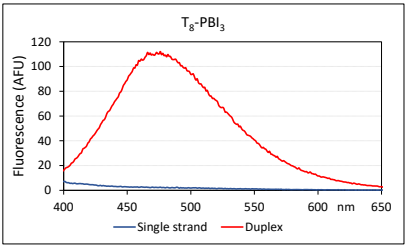
Another distinctive feature of the PBI_3 moiety is its fluorescence properties. Its fluorescence efficiency increases significantly upon binding with a double-stranded DNA. In the case of a PBI_3-oligonucleotide conjugate, fluorescence increase is triggered by the conjugate hybridization with a complementary target. In principle, this allows for a very simple hybridization probe design that requires no additional fluorophore or a quencher.

Optical properties of conjugated PBI_3

Absorbance maximum*	358 nm
Emission maximum**	473 nm
Extinction coefficient (260 nm)	50,000 $M^{-1}cm^{-1}$***
Extinction coefficient (358 nm)	82,700 $M^{-1}cm^{-1}$

**Measured for T_8-PBI_3 conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***Measured for 200 nM T_8-PBI_3 conjugate (10 mM Tris-HCl pH 8.9, 40 mM NaCl, 5 mM $MgCl_2$) at 20°C in the presence of 400 nM A_8C complement*
****PBI_3 contribution only*



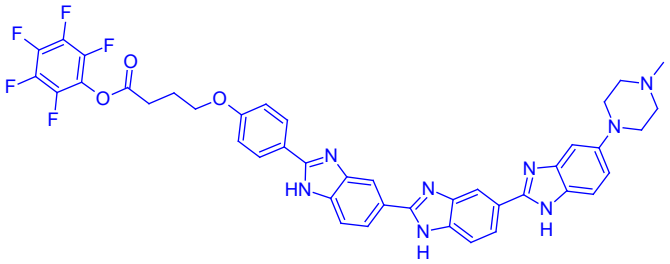


Available PBI_3 MGB products	**Product number**
PBI_3 MGB PFP ester	M830795
PBI_3 MGB CPG	M830796
PBI_3 MGB Polystyrene****	M830797 (bulk), M100243 (columns)

*****Suitable for ABI 3900 DNA synthesizer*

PBI_3 MGB PFP ester

Product number: M830795



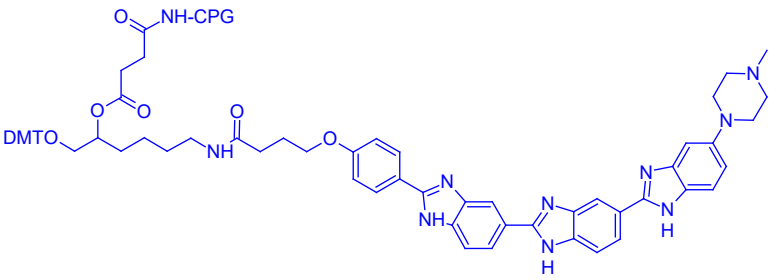
Formula	$C_{42}H_{33}F_5N_8O_3$
Molecular weight (reagent)	792.76 Da
Molecular weight (incorporated)	608.69 Da
Purity* (reverse phase HPLC)	≥90%
Structure identity test	^{1}H NMR
Recommended coupling conditions	See conjugation protocol
Deprotection conditions	N/A
Recommended storage conditions/Stability	≤ -10°C, dry/ 1 year
Available documents	CoA, conjugation protocols

**Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.*

Catalog number	Unit size
M830795	bulk
M830795-1MG	1 mg
M830795-10MG	10 mg

PBI_3 MGB CPG

Product number: M830796

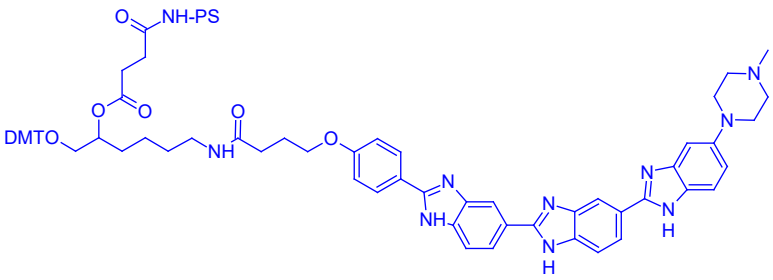


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	803.84 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥55%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830796	bulk
M830796-100MG	100 mg
M830796-250MG	250 mg
M830796-1000MG	1 g

PBI_3 MGB Polystyrene

Product numbers: M830797 (bulk), M100243 (columns)



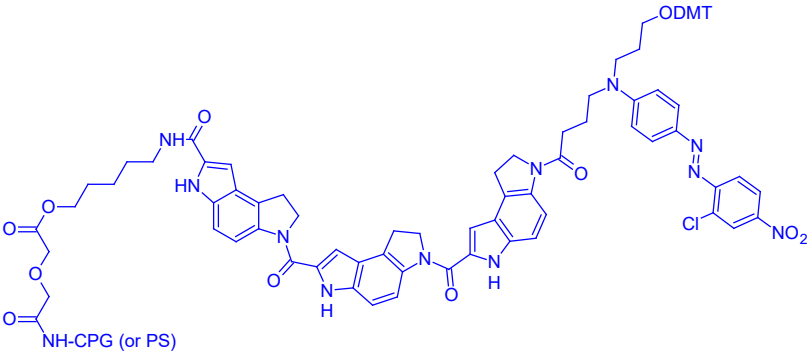
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	803.84 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥55%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830797	bulk
M100243-P3	Pack of 3 columns*
M100243-P10	Pack of 10 columns*
M100243-P30	Pack of 30 columns*
M100243-P100	Pack of 100 columns*

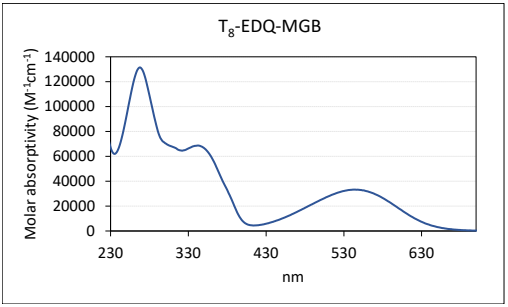
**ABI 3900 type columns, ~200 nmol/column*

11. Eclipse Dark Quencher-MGB synthesis supports

Acronym: EDQ-MGB



EDQ-MGB CPG and polystyrene supports offer the most straightforward way of preparing MGB TaqMan and MGB Eclipse probes (Figure 13A and Figure 13B). When used in combination with 3'-nucleoside phosphoramidites and a fluorophore phosphoramidite at the last step of the synthesis, the supports will yield a 5'-fluorophore, 3'-EDQ-MGB probe, which is the essence of the MGB TaqMan probe's structure. Alternatively, the use of 5'-nucleoside phosphoramidites will afford a 3'-fluorophore, 5'-EDQ-MGB probe known as MGB Eclipse. MGB TaqMan probes generate fluorescence signal during PCR amplification via 5'-exonuclease degradation in the presence of complementary targets. MGB Eclipse probes, on the other hand, are 5'-exonuclease resistant and remain annealed to complementary targets to generate fluorescent signal. Both types of probes benefit from the presence of the duplex-stabilizing MGB moiety which allows for shorter probes with improved mismatch discrimination and reduced background fluorescence.



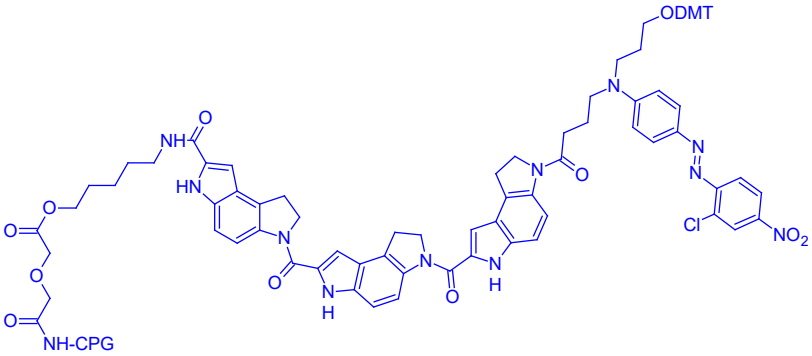
Absorbance maximum*	342 nm, 545 nm
Emission maximum*	N/A
Extinction coefficient (260 nm)	55,700 $M^{-1}cm^{-1}$**
Extinction coefficient (342 nm)	68,700 $M^{-1}cm^{-1}$
Extinction coefficient (545 nm)	33,300 $M^{-1}cm^{-1}$

**Measured for T_8-EDQ-MGB conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***EDQ-MGB contribution only*

Available EDQ-MGB synthesis supports	**Product number**
EDQ-MGB Polystyrene	M830385 (bulk), M130008 (columns)
EDQ-MGB CPG (500 Å)	M830394
EDQ-MGB CPG (1000 Å)	M830394-1000

EDQ-MGB CPG

Product number: M830394 (500 Å), M830394-1000 (1000 Å)

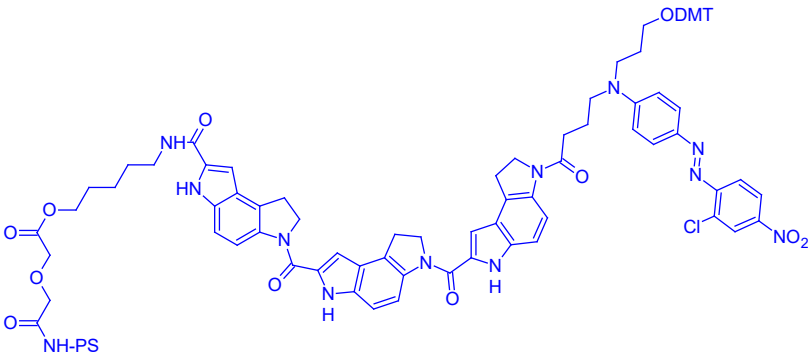


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1120.54 Da
Capacity by DMT loading	≥15 µmol/g (1000 Å) ≥30 µmol/g (500 Å)
Pore size	~500 Å and ~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830394	bulk
M830394-100MG	100 mg
M830394-250MG	250 mg
M830394-1000MG	1 g
M830394-1000	bulk
M830394-1000-100MG	100 mg
M830394-1000-250MG	250 mg
M830394-1000-1000MG	1 g

EDQ-MGB Polystyrene

Product number: M830385 (bulk), M130008 (columns)



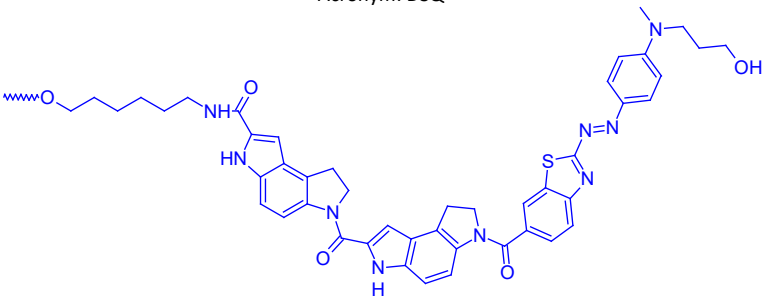
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1120.54 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830385	bulk
M130008-P3	Pack of 3 columns*
M130008-P10	Pack of 10 columns*
M130008-P30	Pack of 30 columns*
M130008-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

12. Duplex-stabilizing quencher

Acronym: DSQ



DSQ is a single molecule that acts as an MGB and a universal fluorescence quencher at the same time. It was designed by converting a part of the original $CDPI_3$ MGB molecule into a quencher without compromising the MGB duplex-stabilizing properties. DSQ was developed to improve MGB TaqMan probe chemistry. It has a number of advantages over the EDQ-MGB chemistry. It eliminates the need for a separate MGB and quencher and streamlines probe manufacturing. More importantly, DSQ improves DNA duplex stabilization (Figure 16), fluorescence quenching (Figure 15 and Figure 17) and signal-to-background ratio (Figure 17).

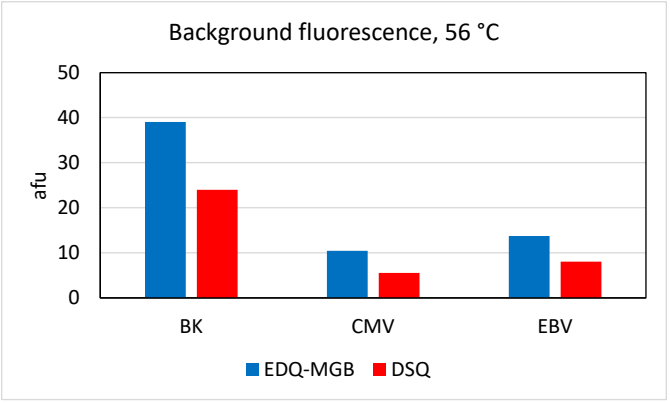


Figure 15 Comparison of background fluorescence for unhybridized FAM-labeled EDQ-MGB and DSQ probes. Examples include probes specific for BK virus (BK), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV).

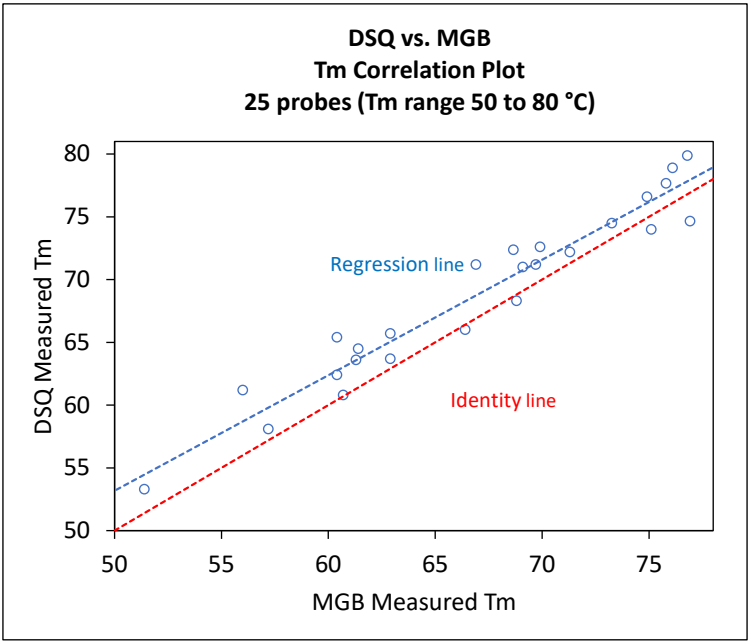


Figure 16 Comparison of melting temperatures of DSQ and EDQ-MGB probes.

On average, DSQ-probe/target duplexes are 2°C (range -2 to +5°C) more stable than the respective MGB-probe/target duplexes (Figure 16).

When compared to MGB TaqMan, DSQ TaqMan probes show a 20-50% reduction of baseline fluorescence, comparable amplification signals (and Ct values) and 10-100% increase of signal-to-baseline ratio (Figure 17).

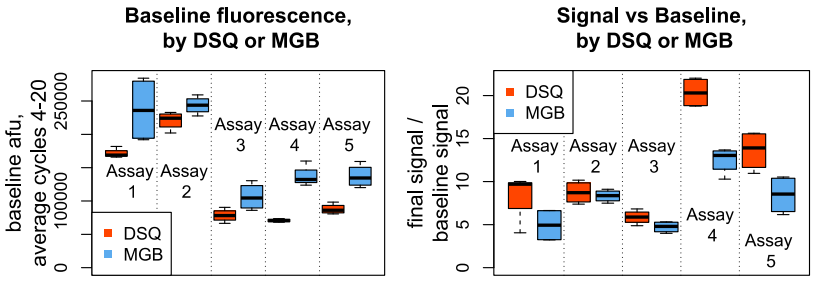
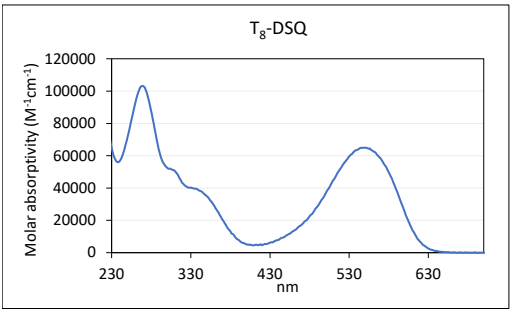


Figure 17 Comparison of real-time PCR baseline fluorescence and signal-to-baseline ratios of DSQ- and EDQ-MGB-modified probes.

Optical properties of conjugated DSQ

Absorbance maximum*	330 nm, 549 nm
Emission maximum*	N/A
Extinction coefficient (260 nm)	25,000 $M^{-1}cm^{-1}$**
Extinction coefficient (330 nm)	39,500 $M^{-1}cm^{-1}$
Extinction coefficient (549 nm)	65,000 $M^{-1}cm^{-1}$

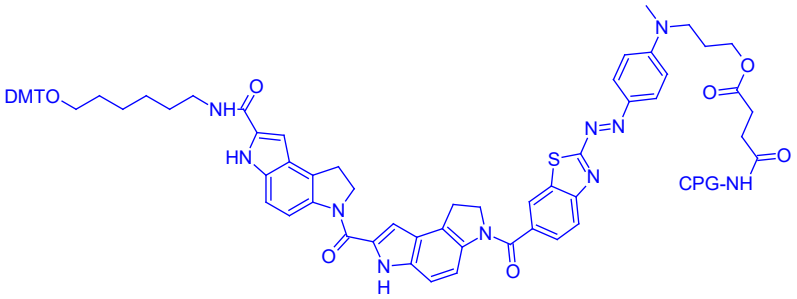
**Measured for T_8-DSQ conjugate (50 mM Tris-HCl pH 8.5, 20°C)*
***DSQ contribution only*



Available DSQ products	Product number
DSQ Polystyrene	M830756 (bulk), M100501 (columns)
DSQ CPG	M830762

DSQ CPG

Product number: M830762

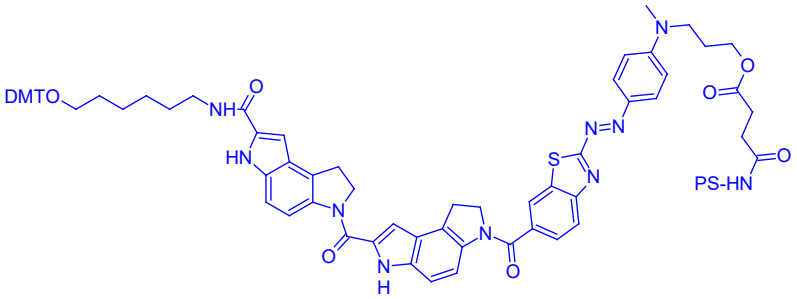


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	899.95 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830762	bulk
M830762-100MG	100 mg
M830762-250MG	250 mg
M830762-1000MG	1 g

DSQ Polystyrene

Product number: M830756 (bulk), M100501 (columns)

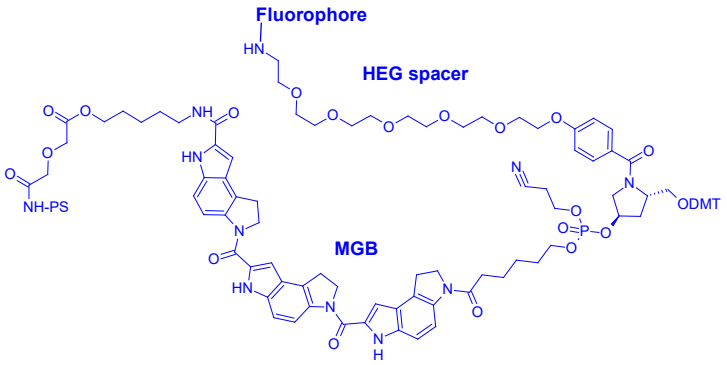


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	899.95 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830756	bulk
M100501-P3	Pack of 3 columns*
M100501-P10	Pack of 10 columns*
M100501-P30	Pack of 30 columns*
M100501-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

13. Fluorophore-MGB ($CDPI_3$) Polystyrene supports

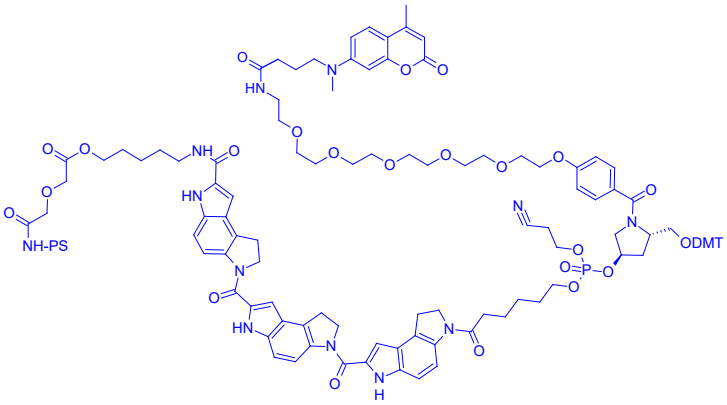


Fluorophore-MGB polystyrene supports have been developed for simultaneous incorporation of a fluorophore and the MGB residue either at the 3' or 5' end of an oligonucleotide. When used in combination with 5'-nucleoside phosphoramidites and a non-fluorescent quencher, these supports yield 5'-fluorophore-MGB, 3'-quencher oligonucleotide probes known as MGB Pleiades probes (Figure 13C). MGB Pleiades are hybridization-triggered fluorogenic probes, which are resistant to 5'-exonuclease degradation and thus suitable for post-PCR melting curve analysis. Owing to the efficiency of a dual quenching mechanism, mediated by both the MGB and a non-fluorescent quencher, Pleiades probes possess a very low background fluorescence in the absence of a target. Fluorescence is restored upon probe hybridization with complementary target with excellent signal-to-background ratios. Additionally, fluorophores are separated from the MGB group by the hexa-ethylene glycol (HEG) spacer for improved hybridization-dependent fluorescence.

Available Fluorophore MGB Polystyrenes	Product number
EB380-MGB Polystyrene	M830740 (bulk), M100431 (columns)
FAM-MGB Polystyrene	M830693 (bulk), M100185 (columns)
AP525-MGB Polystyrene	M830692 (bulk), M100186 (columns)
AP559-MGB Polystyrene	M830689 (bulk), M100181 (columns)
AP593-MGB Polystyrene	M830691 (bulk), M100135 (columns)
AP639-MGB Polystyrene	M830738 (bulk), M100293 (columns)
AP642-MGB Polystyrene	M830200 (bulk), M100153 (columns)
AP662-MGB Polystyrene	M830695 (bulk), M100250 (columns)
AP690-MGB Polystyrene	M830754 (bulk), M100499 (columns)

EB380-MGB Polystyrene

Product number: M830740 (bulk), M100431 (columns)



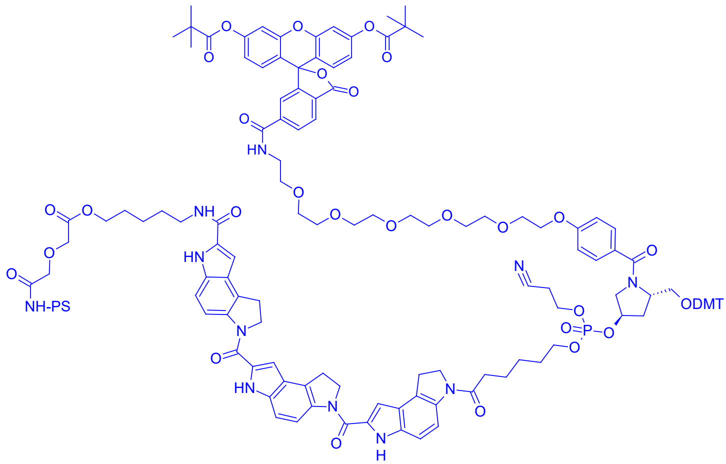
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1651.68 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥75%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830740	bulk
M100431-P3	Pack of 3 columns*
M100431-P10	Pack of 10 columns*
M100431-P30	Pack of 30 columns*
M100431-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

FAM-MGB Polystyrene

Product number: M830693 (bulk), M100185 (columns)



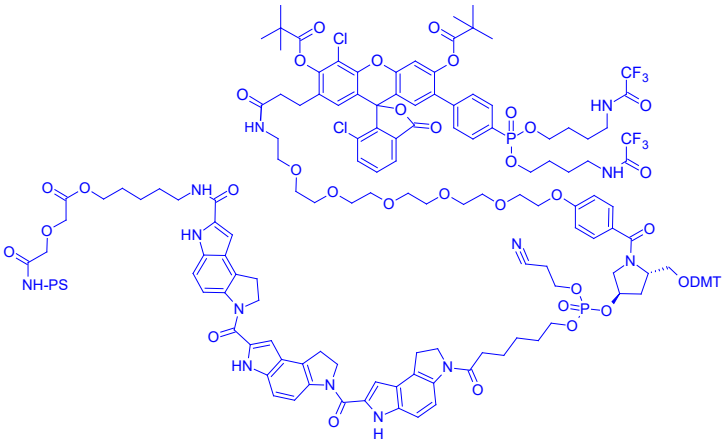
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1752.69 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥75%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 8 years
Available documents	Certificate of analysis

Catalog number	**Unit size**
M830693	bulk
M100185-P3	Pack of 3 columns*
M100185-P10	Pack of 10 columns*
M100185-P30	Pack of 30 columns*
M100185-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP525-MGB Polystyrene

Product number: M830692 (bulk), M100186 (columns)



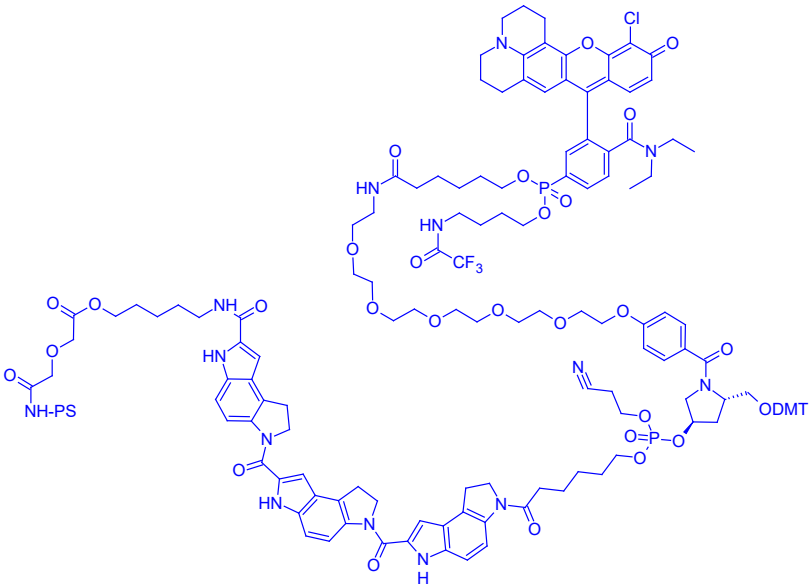
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2076.84 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥75%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830692	bulk
M100186-P3	Pack of 3 columns*
M100186-P10	Pack of 10 columns*
M100186-P30	Pack of 30 columns*
M100186-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP559-MGB Polystyrene

Product number: M830689 (bulk), M100181 (columns)



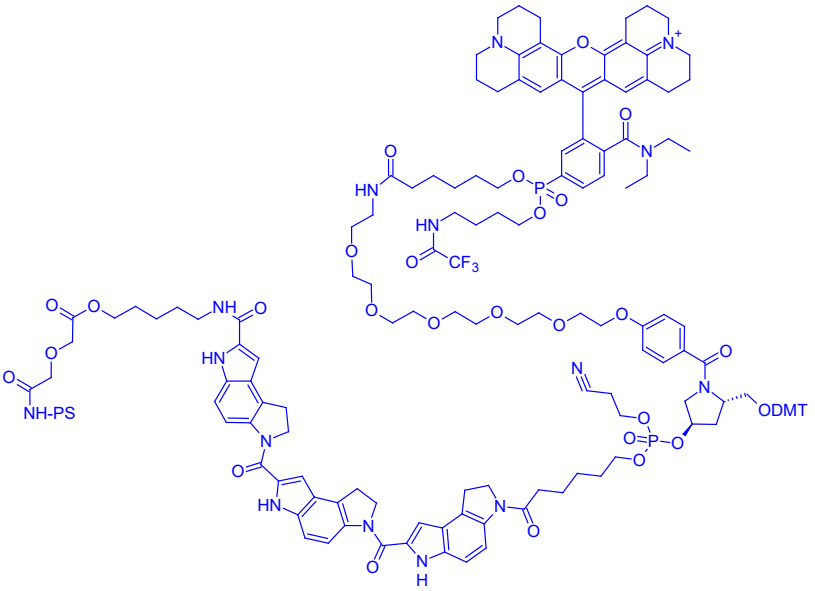
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2071.52 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830689	bulk
M100181-P3	Pack of 3 columns*
M100181-P10	Pack of 10 columns*
M100181-P30	Pack of 30 columns*
M100181-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP593-MGB Polystyrene

Product number: M830685 (bulk), M100135 (columns)



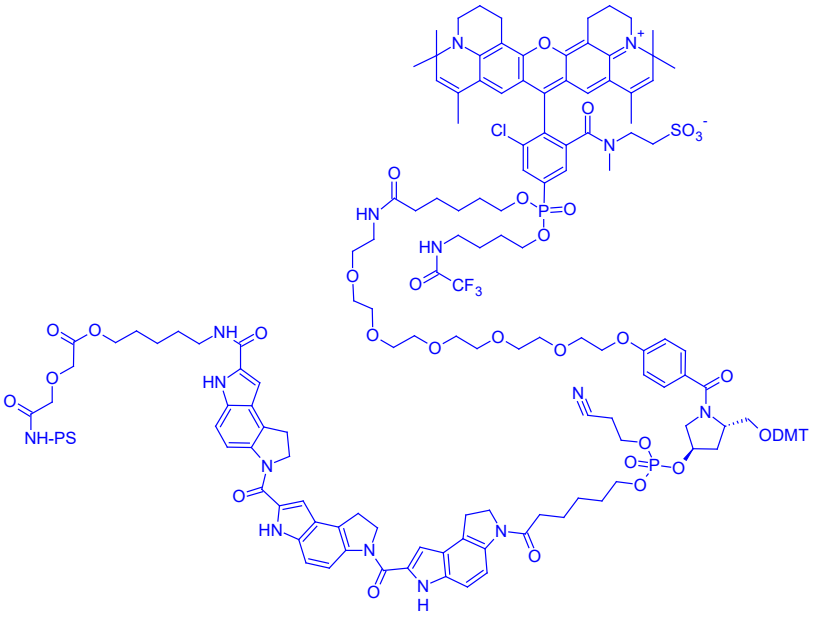
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2116.22 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 4 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830685	bulk
M100135-P3	Pack of 3 columns*
M100135-P10	Pack of 10 columns*
M100135-P30	Pack of 30 columns*
M100135-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP639-MGB Polystyrene

Product number: M830738 (bulk), M100293 (columns)



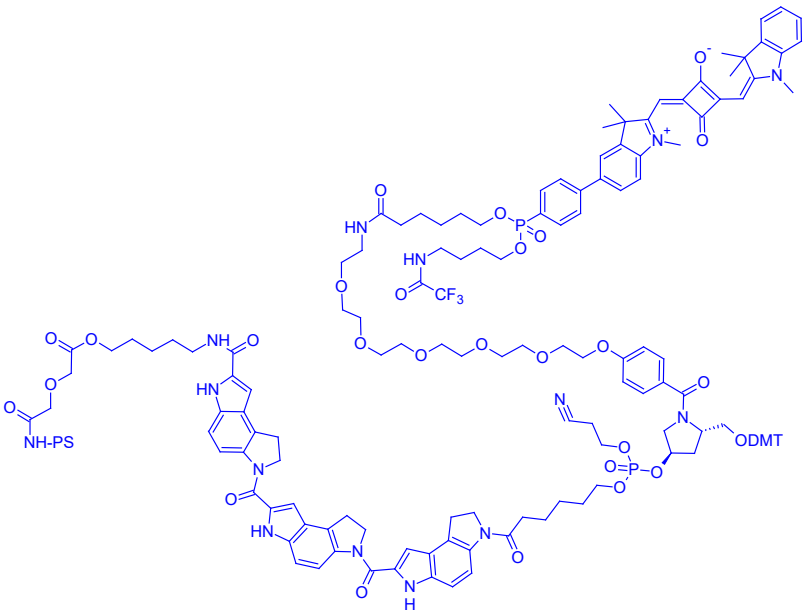
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2296.82 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 6 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830738	bulk
M100293-P3	Pack of 3 columns*
M100293-P10	Pack of 10 columns*
M100293-P30	Pack of 30 columns*
M100293-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP642-MGB Polystyrene

Product number: M830200 (bulk), M100153 (columns)



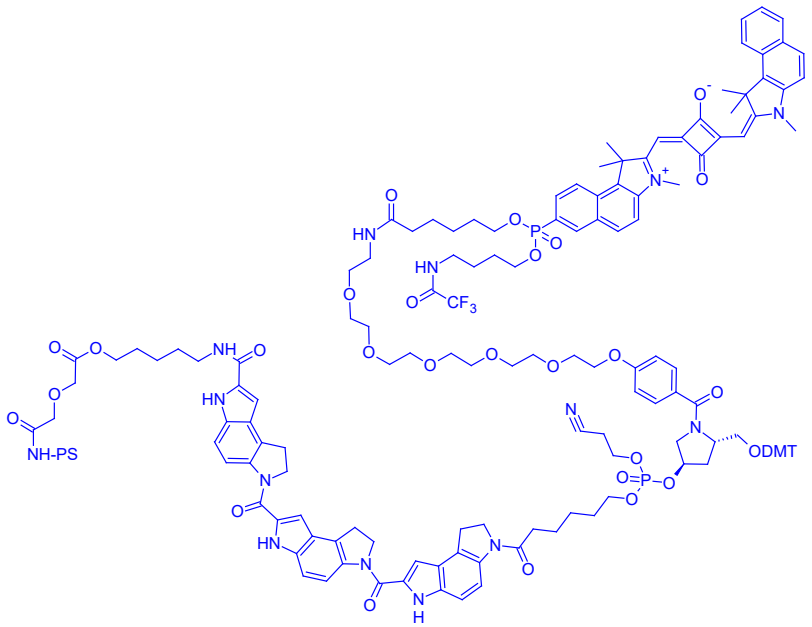
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2071.14 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 8 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830200	bulk
M100153-P3	Pack of 3 columns*
M100153-P10	Pack of 10 columns*
M100153-P30	Pack of 30 columns*
M100153-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP662-MGB Polystyrene

Product number: M830695 (bulk), M100250 (columns)



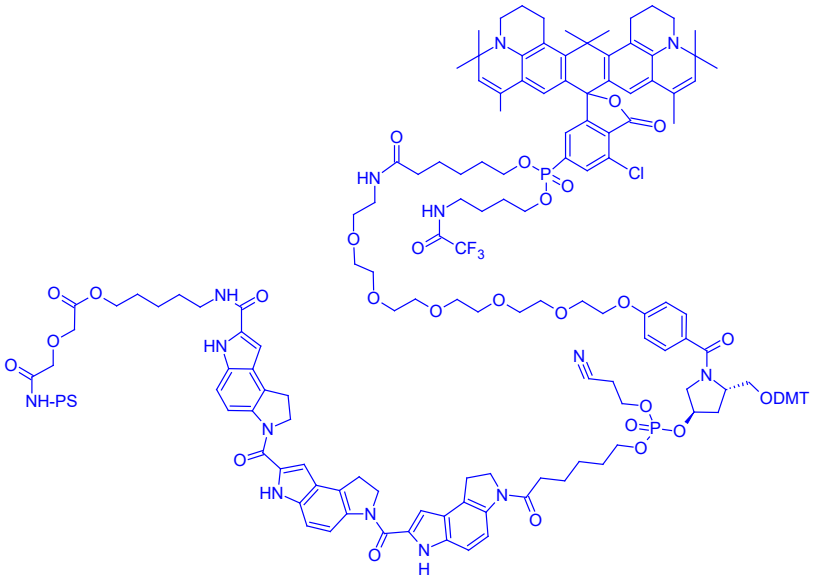
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2095.16 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 9 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830695	bulk
M100250-P3	Pack of 3 columns*
M100250-P10	Pack of 10 columns*
M100250-P30	Pack of 30 columns*
M100250-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

AP690-MGB Polystyrene

Product number: M830754 (bulk), M100499 (columns)



Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	2201.75 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥65%
Recommended coupling conditions	N/A
Recommended deprotection conditions	$^{t}BuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 3 years
Available documents	Certificate of analysis

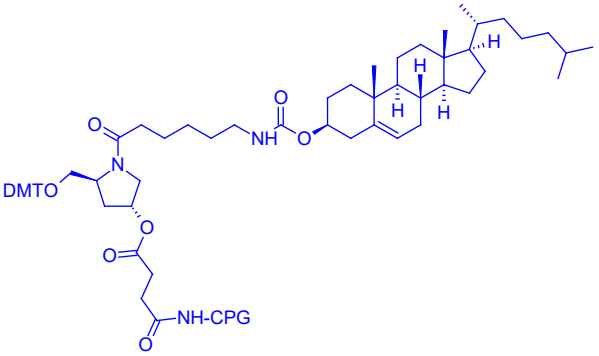
Catalog number	Unit size
M830754	bulk
M100499-P3	Pack of 3 columns*
M100499-P10	Pack of 10 columns*
M100499-P30	Pack of 30 columns*
M100499-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

14. Lipophilic oligonucleotides

Cholesterol CPG

Product number: M830761

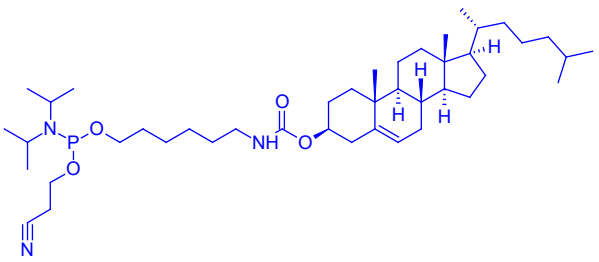


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	704.91 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830761	bulk
M830761-100MG	100 mg
M830761-250MG	250 mg
M830761-1000MG	1 g

Cholesterol Phosphoramidite

Product number: M830785; CAS #: 143723-64-2



Formula	$C_{43}H_{76}N_3O_4P$
Molecular weight (reagent)	730.06 Da
Molecular weight (incorporated)	591.80 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

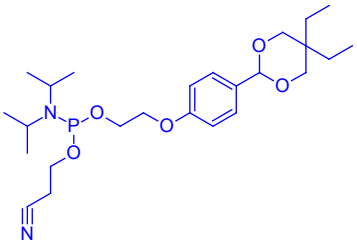
Catalog number	Unit size
M830785	bulk
M830785-50UMOL	50 µmol
M830785-100UMOL	100 µmol
M830785-250MG	250 mg
M830785-1000MG	1 g

15. Amine-modifiers, Linkers, and Spacers

Product number	Product name	Functional group introduced
M830547	Benzaldehyde Phosphoramidite	Aromatic aldehyde
M830758	DMT-Hexanol Diglycolate-CPG	C6-Primary hydroxyl
M126137 (bulk), M100457 (colunms)	DMT-Hexanol-Diglycolate-Polystyrene	C6-Primary hydroxyl
M830780	TFA-Aminopropyl-dU 3'-Phosphoramidite	C3-Primary amine at 5-position of Uracil
M830715	TFA-Aminopropyl-dU 5'-Phosphoramidite	C3-Primary amine at 5-position of Uracil
M830784	TFA-Aminopropyl-Super G 3'-Phosphoramidite	C3-Primary amine of Super G base
M830767	Fmoc-HEG Phosphoramidite	Primary amine with a 17 atom spacer
M830769	Fmoc-Hydroxyprolinol Phosphoramidite	Secondary amine
M830768	Fmoc-Hydroxyprolinol CPG	Secondary amine
M830770 (bulk), M100510 (columns)	Fmoc-Hydroxyprolinol Polystyrene	Secondary amine
M830765	Fmoc-Aminohexanediol CPG	C6-Primary amine
M830766 (bulk), M100511 (columns)	Fmoc-Aminohexanediol Polystyrene	C6-Primary amine
M830798 (bulk), M100508 (columns)	Fmoc-HEG-MGB Polystyrene	MGB and Primary amine with a 17 atom spacer

Benzaldehyde Phosphoramidite

Product number: M830547; CAS #: 433684-36-7



Benzaldehyde phosphoramidite is suitable for the introduction of an aromatic aldehyde at the 5' or 3' end of an oligonucleotide. The cyclic acetal provides a hydrophobic handle for reverse phase HPLC purification and can be removed after HPLC by treatment with 80% acetic acid. Alternatively, the acetal protection is removed during the standard on-line detritylation step.

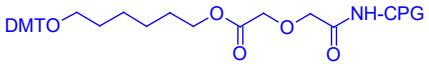
Aromatic aldehydes are mild electrophiles, which are capable of forming Schiff base type adducts with various nucleophiles, e.g., amines, hydrazines or hydrazides (Ref. 33).

Formula	$C_{25}H_{41}N_2O_5P$
Molecular weight (reagent)	480.58 Da
Molecular weight (incorporated)	228.13 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^1H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830547	bulk
M830547-50UMOL	50 µmol
M830547-100UMOL	100 µmol
M830547-250MG	250 mg
M830547-1000MG	1 g

DMT-Hexanol Diglycolate CPG

Product number: M830758

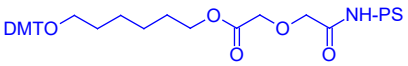


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	180.13 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830758	bulk
M830758-100MG	100 mg
M830758-250MG	250 mg
M830758-1000MG	1 g

DMT-Hexanol Diglycolate Polystyrene

Product number: M126137 (bulk), M100457 (columns)



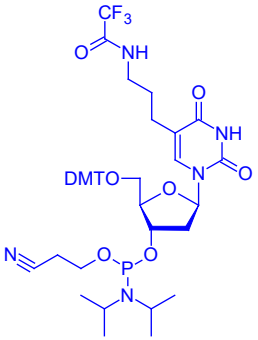
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	180.13 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M126137	bulk
M100457-P3	Pack of 3 columns*
M100457-P10	Pack of 10 columns*
M100457-P30	Pack of 30 columns*
M100457-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

TFA-Aminopropyl-dU 3'-Phosphoramidite

Product number: M830780; CAS #: 134140-86-6

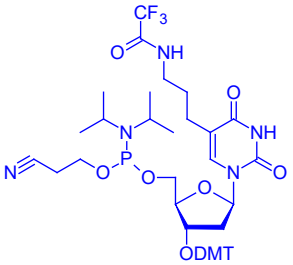


Formula	$C_{44}H_{53}F_3N_5O_9P$
Molecular weight (reagent)	883.89 Da
Molecular weight (incorporated)	347.26 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^1H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N',N'-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830780	bulk
M830780-50UMOL	50 µmol
M830780-100UMOL	100 µmol
M830780-250MG	250 mg
M830780-1000MG	1 g

TFA-Aminopropyl-dU 5'-Phosphoramidite

Product number: M830715; CAS #: 1146714-75-1

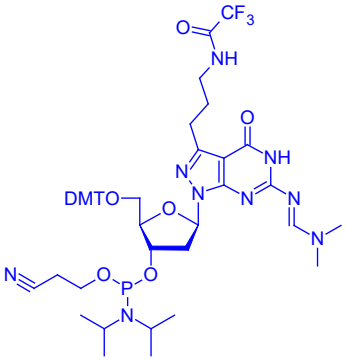


Formula	$C_{44}H_{53}F_3N_5O_9P$
Molecular weight (reagent)	883.89 Da
Molecular weight (incorporated)	347.26 Da
Purity (reverse phase HPLC)	≥80%
Purity of test oligo (reverse phase HPLC)	≥80%
Identity	^1H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N',N'-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830715	bulk
M830715-50UMOL	50 µmol
M830715-100UMOL	100 µmol
M830715-250MG	250 mg
M830715-1000MG	1 g

TFA-Aminopropyl-Super G 3'-Phosphoramidite

Product number: M830784; CAS #: 872840-74-9

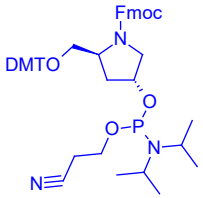


Formula	$C_{48}H_{59}F_3N_9O_8P$
Molecular weight (reagent)	978.01 Da
Molecular weight (incorporated)	386.30 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^1H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N',N'-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830784	bulk
M830784-50UMOL	50 µmol
M830784-100UMOL	100 µmol
M830784-250MG	250 mg
M830784-1000MG	1 g

Fmoc-Hydroxyprolinol Phosphoramidite

Product number: M830769; CAS #: 2378004-37-4

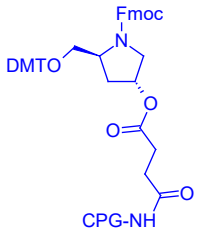


Formula	$C_{50}H_{56}N_3O_7P$
Molecular weight (reagent)	841.97 Da
Molecular weight (incorporated)	179.11 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	Pre-treatment with 5% N,N,N',N'-tetramethylguanidine in CH_3CN followed by 30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830769	bulk
M830769-50UMOL	50 µmol
M830769-100UMOL	100 µmol
M830769-250MG	250 mg
M830769-1000MG	1 g

Fmoc-Hydroxyprolinol CPG

Product number: M830768

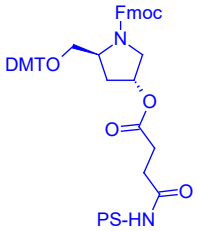


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	179.11 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830768	bulk
M830768-100MG	100 mg
M830768-250MG	250 mg
M830768-1000MG	1 g

Fmoc-Hydroxyprolinol Polystyrene

Product number: M830770 (bulk), M100510 (columns)



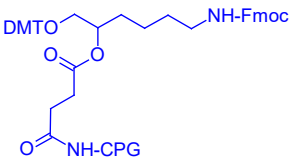
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	179.11 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830770	bulk
M100510-P3	Pack of 3 columns*
M100510-P10	Pack of 10 columns*
M100510-P30	Pack of 30 columns*
M100510-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Fmoc-Aminohexanediol CPG

Product number: M830765

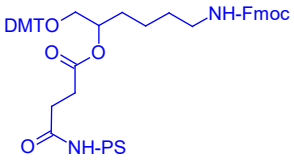


Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	195.15 Da
Capacity by DMT loading	≥15 µmol/g
Pore size	~1000 Å
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ TBD
Available documents	Certificate of analysis

Catalog number	Unit size
M830765	bulk
M830765-100MG	100 mg
M830765-250MG	250 mg
M830765-1000MG	1 g

Fmoc-Aminohexanediol Polystyrene

Product number: M830766 (bulk), M100511 (columns)



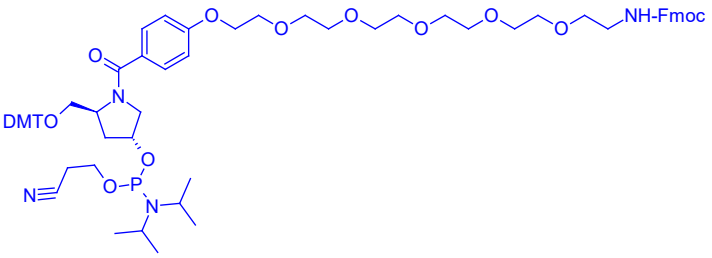
Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	195.15 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830766	bulk
M100511-P3	Pack of 3 columns*
M100511-P10	Pack of 10 columns*
M100511-P30	Pack of 30 columns*
M100511-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Fmoc-HEG Phosphoramidite

Product number: M830767; CAS #: 2378004-40-9

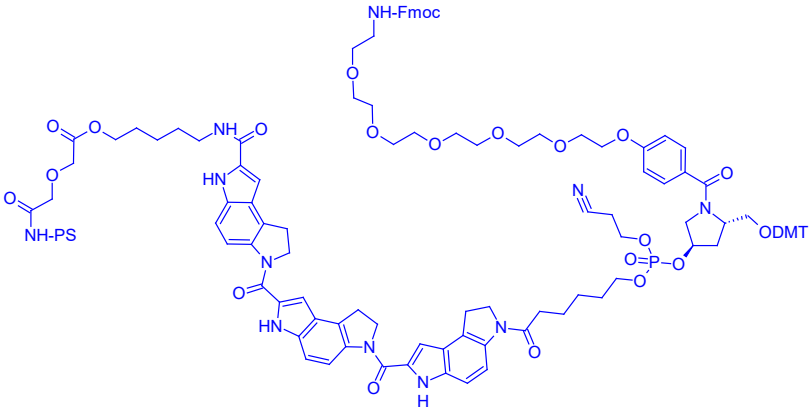


Formula	$C_{69}H_{85}N_4O_{14}P$
Molecular weight (reagent)	1225.41 Da
Molecular weight (incorporated)	562.54 Da
Purity (reverse phase HPLC)	≥90%
Purity of test oligo (reverse phase HPLC)	≥85%
Identity	^{1}H, ^{31}P NMR
Recommended coupling conditions	Double phosphoramidite addition and extended coupling time
Recommended deprotection conditions	30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	≤ -10°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830767	bulk
M830767-50UMOL	50 µmol
M830767-100UMOL	100 µmol
M830767-250MG	250 mg
M830767-1000MG	1 g

Fmoc-HEG-MGB Polystyrene

Product number: M830798 (bulk), M100508 (columns)



Formula	N/A
Molecular weight (reagent)	N/A
Molecular weight (incorporated)	1394.39 Da
Capacity by DMT loading	≥10 µmol/g
Purity of test oligo (reverse phase HPLC)	≥85%
Recommended coupling conditions	N/A
Recommended deprotection conditions	30% NH_4OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
Recommended storage conditions/Stability	2-8°C, dry/ 5 years
Available documents	Certificate of analysis

Catalog number	Unit size
M830798	bulk
M100508-P3	Pack of 3 columns*
M100508-P10	Pack of 10 columns*
M100508-P30	Pack of 30 columns*
M100508-P100	Pack of 100 columns*

**ABI 3900 type columns, ~200 nmol/column*

Appendix A. Determination of the relative hydrophobicities of conjugated dyes using C18 column chromatography

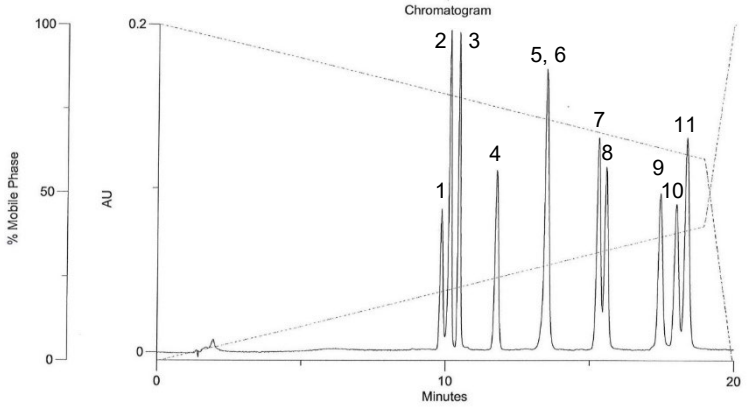


Figure 18 HPLC analysis of a mixture of fluorescent dye calibrators.

1 - T_8-RR (M830731); 2 - T_8-AP525 (M300587); 3 - T_8-FAM-HEG (M300586); 4 - T_8-EB380-HEG (M300585); 5 - T_8-YY (M830730); 6 - T_8-AP559 (M301430); 7 - T_8-AP570 (M830700) ; 8 - T_8-AP593 (M300392) ; 9 - T_8-AP642 (M300589) ; 10 - T_8-AP662 (M830724) ; 11 - T_8-AP680 (M300753).

Column and HPLC conditions: C18 Luna (4.6x100 mm), Phenomenex (00D-4041-E0). Sample loop – 20 µL. Flow rate – 1ml/min, Solvent A – 0.1 M triethylammonium acetate pH 7.5. Solvent B – CH_3CN. Gradient – 0-40% B in 19 min. Detection – 260 nm.

Product number	Product name	Retention (min)	Hydrophobicity (% CH_3CN)
M300585	T_8-EB380-HEG Dye Calibrator	11.77	22.0
M300586	T_8-FAM-HEG Dye Calibrator	10.46	19.2
M302132	T8-GG Dye Calibrator	11.53	21.5
M300587	T_8-AP525 Dye Calibrator	10.14	18.6
M830730	T_8-YY Dye Calibrator	13.49	25.6
M301430	T_8-AP559 Dye Calibrator	13.49	25.6
M830700	T_8-AP570 Dye Calibrator	15.30	29.4
M830731	T_8-RR Dye Calibrator	9.85	17.9
M300392	T_8-AP593 Dye Calibrator	15.56	29.9
M301715	T_8-AP639 Dye calibrator	19.14	37.5
M300589	T_8-AP642 Dye Calibrator	17.43	33.9
M830724	T_8-AP662 Dye Calibrator	17.99	35.0
M300753	T_8-AP680 Dye Calibrator	18.35	35.8
M302123	T_8-AP690 Dye Calibrator	15.81	30.5

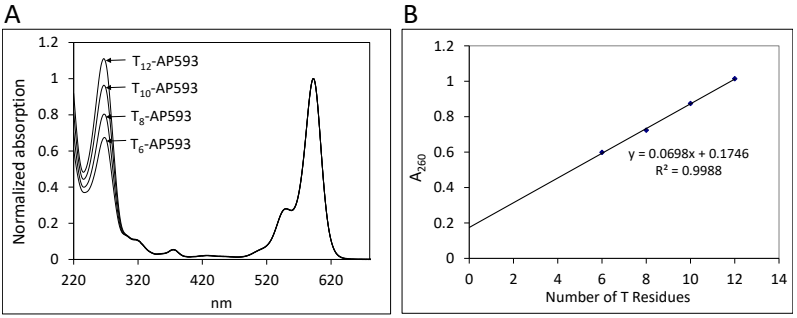
Appendix B. Determination of molar extinction coefficients of conjugated fluorophores and quenchers

The determination of extinction coefficient for a conjugated dye may be challenging due to the sensitivity of absorption and fluorescence spectra to the dye's environment and potentially complex dye-conjugate interactions. In the case of oligonucleotide conjugates, for instance, dyes may interact with nucleobases as well as other conjugated moieties. To take into account these effects, an optimal measurement procedure should employ a dye-oligonucleotide conjugate. However, determination of the dye-oligonucleotide concentration by pure conjugate mass, which is required for the direct extinction coefficient measurement, is often impractical. We have overcome this problem by using a concentration-independent approach which utilizes the known extinction coefficients of oligothymidylates and the dye absorption as an internal reference. In this approach, a series of dye-oligothymidylate conjugates is prepared with a variable number of thymidine units. The molar extinction coefficient for oligothymidylate can be described by a simple formula $E_{260} = 8100n + 600$, where n is the number of thymidine residues. Assuming that the dye conjugation does not affect this dependence, the combined extinction coefficient for dye-oligoT conjugate can be represented as E_{260} (conjugate) $= 8100n + 600 + E_{260}$(dye). To obtain E_{260}(dye), one must measure A_{260} (260 nm absorption), $A_{\lambda max}$(dye) (absorption at the dye maximum) and plot A_{260}(conjugate)/$A_{\lambda max}$(dye) values over the number of thymidine units. These data are then analyzed by linear regression to generate a line of best fit and its corresponding equation $y = ax + b$. Since T oligomers' slope parameter a equals 8100, the equation can be rewritten as $y = 8100n + b/a8100$.

The procedure is illustrated in Figure 19. In this example, the described approach was used to determine an extinction coefficient for a T_n-conjugated AP593 dye.

It should be noted that the success of this method relies on several assumptions and requirements listed below:

1) Dye-oligonucleotide interactions are independent on the length of the oligomers;
2) All conjugates must be carefully purified to ensure accurate A_{260} measurements;
3) The linear fit of all data points should have a high (>0.99) R^2 value to ensure accurate y-intercept determination.

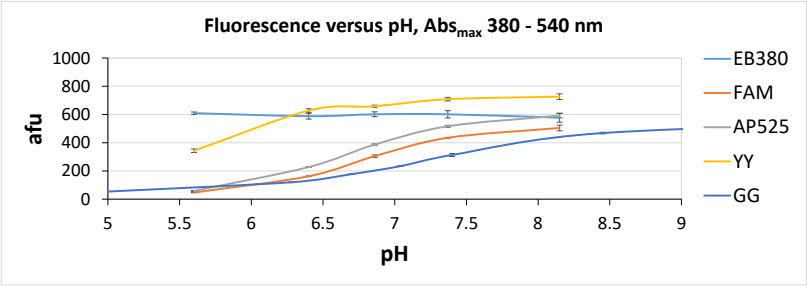


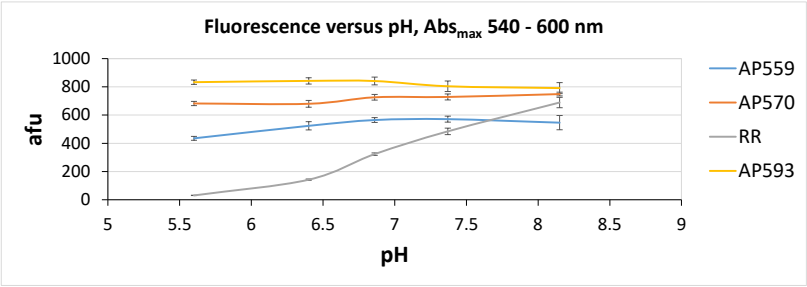
C E_{260}(dye) = 0.1746 * 8100 / 0.0698 – 600 = 19661.6 ($M^{-1}cm^{-1}$)

Figure 19 Extinction coefficient (E_{260}) determination for the AP593 dye conjugated to oligothymidylates.

(A) Normalized (at 593 nm) absorption spectra of T_{12}-, T_{10}-, T_8- and T_6-AP593 conjugates. (B) Line of best fit (with equation and R^2 value) for the normalized A_{260} values versus number of thymidine units. (C) Calculation of the E_{260}(dye) coefficient using the parameters a *and* b *from the best fit linear equation. The y-intercept (*b/a*8100) of this line represents E_{260}(dye) + 600. Therefore, E_{260}(dye) =* b*8100/*a *– 600.*

Appendix C. Effect of pH on dye fluorescence





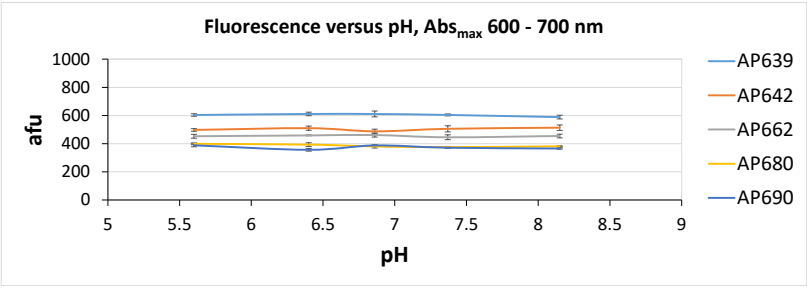
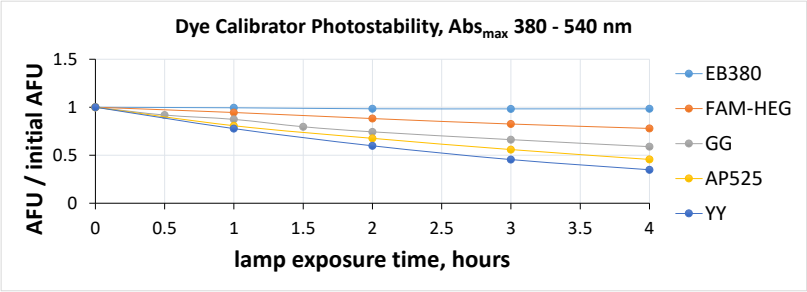
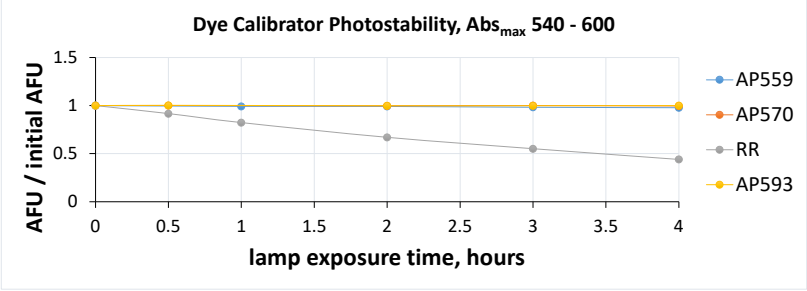


Figure 20 Effect of pH on fluorescence signal for various T_8-Dye calibrators.

Experimental conditions: Instrument: Varian Cary Eclipse; 5 nm slit width; Buffer: 40 mM sodium phosphate; Temperature: 20 ℃.

Appendix D. Photostability of fluorescent dyes





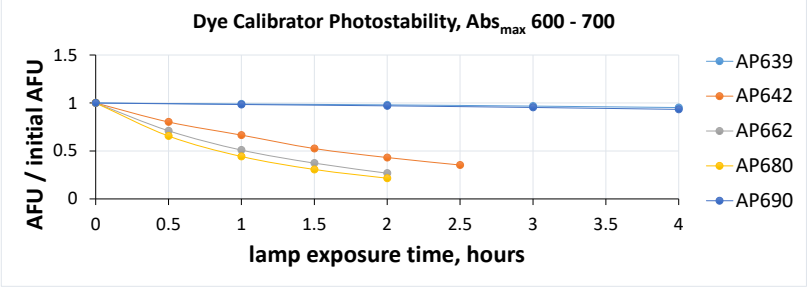


Figure 21 Relative photostability of fluorescent dyes.

500 nM dye calibrator solutions in TE (pH 8.0) were exposed to a 250 W incandescent bulb at a distance of 20 cm, using a water bath to maintain temperature at 20-35°C.

Note: AP559 and AP570 data partly obscured by AP593; AP639 data partly obscured by AP690.

Appendix E. Fluorescence temperature dependence

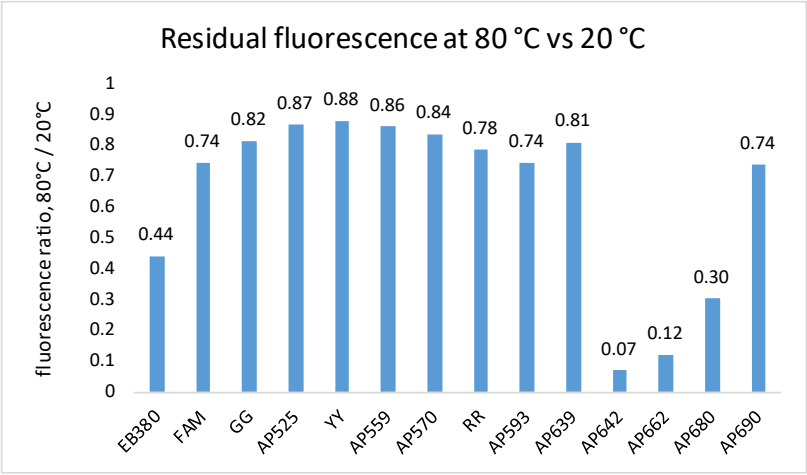


Figure 22 Effect of temperature increase (from 20°C to 80°C) on dye fluorescence.

Concentration: 100 nM dye calibrator in 50 mM Tris-HCl (pH 8.0)

QUICK REFERENCE GUIDE: FLUORESCENT DYES

Name	Abs Max (nm)	E_{max} ($M^{-1}cm^{-1}$)	Em Max (nm)	Φ	pH sensitivity	Hydro-phobicity*	Photo-stability**
Epoch Blue 380	381	20,000	460	0.24	not sensitive	22	High
6-FAM	496	80,400	517	0.88	pKa~7	19.2	Medium High
Gig Harbor Green	497	93,200	521	0.85	pKa~7.5	21.5	Medium
AquaPhluor 525	527	99,300	549	0.73	pKa~7	18.6	Medium
Yakima Yellow	525	86,500	547	0.57	pKa~6	25.6	Medium
AquaPhluor 559	559	86,500	581	0.64	not sensitive	25.6	High
AquaPhluor 570	570	101,100	592	0.68	not sensitive	29.4	High
Redmond Red	580	64,500	594	0.46	pKa~7.5	17.9	Medium
AquaPhluor 593	593	115,240	613	0.61	not sensitive	29.9	High
AquaPhluor 639	639	129,300	655	0.28	not sensitive	37.5	High
AquaPhluor 642	642	283,200	653	0.13	not sensitive	33.9	Low
AquaPhluor 662	662	241,100	672	0.14	not sensitive	35	Low
AquaPhluor 680	682	221,300	704	0.07	not sensitive	35.8	Low
AquaPhluor 690	689	141,900	709	0.14	not sensitive	30.5	High

*See Appendix A

**See Appendix D

QUICK GUIDE FOR OLIGONUCLEOTIDE SYNTHESIS: PHOSPHORAMIDITES

Phosphoramidite name	Product number	Coupling conditions*		Recommended deprotection conditions	
		# of addns	Wait time	30% NH_4OH/EtOH (17:3 v:v), 16 h at 55°C or 2 h at 70°C	$^tBuNH_2$:MeOH: H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
AP525	M830199	2	Standard	✓	Not recommended
AP525-HEG	M830714	2	Standard	✓	Not recommended
AP559 Internal	M830195	4	Extended	✓	✓
AP559 Terminal	M830698	4	Extended	✓	✓
AP570 Internal	M830675	2	Standard	✓	✓
AP570 Terminal	M830699	2	Standard	✓	✓
AP593	M830736	2	Standard	✓	✓
AP639	M830741	4	Extended	✓	✓
AP690	M830760	2	Standard	Partial lactam formation	✓
Benzaldehyde	M830547	2	Standard	✓	✓
$CDPI_3$ MGB Terminal	M830252	2	Standard	30% NH_4OH/EtOH (3:1; v:v)	✓
Cholesterol	M830785	2	Standard	✓	✓
EB380	M830192	2	Standard	✓	✓
EB380-HEG	M830727	2	Standard	✓	✓
EDQ	M830028	2	Standard	✓	✓
EDQ-Super I 3'-	M830781	2	Standard	✓	✓
FAM	M100011	2	Standard	✓	✓
FAM-dU 3'	M830763	2	Standard	✓	✓
FAM-dU 5'	M830764	2	Standard	✓	✓
FAM-HEG	M830100	2	Standard	✓	✓
FAM-Super I 3'-	M830783	2	Standard	✓	✓
Fmoc-HEG	M830767	2	Standard	✓	✓
Fmoc-hydroxyprolinol	M830769	2	Standard	✓	✓
LQ-380 Internal	M830786	2	Standard	✓	✓
LQ-400 Internal	M830788	2	Standard	✓ Partial dye degradation	✓
NNA A	M830772	2	Standard	✓	✓
NNA C	M830773	2	Standard	✓	✓
NNA G	M830774	2	Standard	✓	✓
NNA T	M830775	2	Standard	✓	✓
p-DNA A	M830776	2	Standard	✓	✓
p-DNA C	M830777	2	Standard	✓	✓
p-DNA G	M830778	2	Standard	✓	✓
p-DNA T	M830779	2	Standard	✓	✓
Pyrene Internal	M830711	2	Standard	✓	✓
Pyrene-Super I- 3'-	M830782	2	Standard	✓	✓
Q-470	M830565	2	Standard	✓	✓
Q-575	M830566	2	Standard	✓ Partial dye degradation	✓

Phosphoramidite name	Product number	Coupling conditions*		Recommended deprotection conditions	
		# of addns	Wait time	30% NH_4OH/EtOH (17:3 v:v), 16 h at 55°C or 2 h at 70°C	$^tBuNH_2$:MeOH: H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
Q-630	M830567	2	Standard	Not recommended	✓
Redmond Red	M830035	2	Standard	✓	✓
Super A 3'-	M830249	2	Standard	✓	✓ Add extra time for multiple incorporations
Super A 5'-	M830250	2	Standard	✓	✓ Add extra time for multiple incorporations
Super G 3'-	M830006	2	Standard	✓	✓
Super G 5'-	M830031	2	Standard	✓	✓
Super I 3'-	M830193	2	Standard	✓ TMG pre-treatment recommended	✓ TMG pre-treatment recommended
Super I 5'-	M830712	2	Standard	✓ TMG pre-treatment recommended	✓ TMG pre-treatment recommended
Super T 3'-	M830040	2	Standard	✓	✓
Super T 5'-	M830041	2	Standard	✓	✓
TFA-aminopropyl-dU 3'	M830780	2	Standard	✓	✓
TFA-aminopropyl-dU 5'	M830715	2	Standard	✓	✓
TFA-aminopropyl-Super G 3'-	M830784	2	Standard	✓	✓
Yakima Yellow	M830552	2	Standard	✓	Not recommended

**Optimized for ABI 3900 DNA Synthesizer. Other instruments' coupling conditions may vary.*

QUICK GUIDE FOR OLIGONUCLEOTIDE SYNTHESIS: SYNTHESIS SUPPORTS

Product name	Product number bulk (b) columns (c)	Recommended deprotection conditions	
		30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C	$^tBuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
AP525 CPG	M830671	✓	Not recommended
AP525 PS	M830672 (b) M100276 (c)	✓	Not recommended
AP525-MGB PS	M830692 (b) M100186 (c)	30% NH_4OH/EtOH (3:1; v:v)	Not recommended
AP559 CPG	M830673	✓	✓
AP559 PS	M830674 (b) M100278 (c)	✓	✓
AP559-MGB PS	M830689 (b) M100181 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
AP570 CPG	M830676	✓	✓
AP570 PS	M830677 (b) M100444 (c)	✓	✓
AP593 CPG	M830208	✓	✓
AP593 PS	M830732 (b) M100257 (c)	✓	✓
AP593-MGB PS	M830685 (b) M100135 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
AP639 CPG	M830757	✓	✓
AP639 PS	M830743 (b) M100440 (c)	✓	✓
AP639-MGB PS	M830738 (b) M100293 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
AP642 CPG	M830190	Not recommended	✓
AP642 PS	M830678 (b) M100279 (c) M100280 (c)	Not recommended	✓
AP642-MGB PS	M830200 (b) M100153 (c)	Not recommended	✓
AP662 CPG	M830191	Not recommended	✓
AP662 PS	M830679 (b) M100271 (c)	Not recommended	✓
AP662-MGB PS	M830695 (b) M100250 (c)	Not recommended	✓
AP680 CPG	M830680	Not recommended	50°C/8 h
AP680 PS	M830681 (b) M100282 (c)	Not recommended	50°C/8 h
AP690 CPG	M830759	Partial lactam formation	✓

Product name	Product number bulk (b) columns (c)	Recommended deprotection conditions	
		30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C	$^tBuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
AP690 PS	M830755 (b) M100500 (c)	Partial lactam formation	✓
AP690-MGB PS	M830754 (b) M100499 (c)	Partial lactam formation	✓
$CDPI_3$ MGB CPG	M830739	30% NH_4OH/EtOH (3:1; v:v)	✓
$CDPI_3$ MGB PS	M830203 (b) M100103 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
Cholesterol CPG	M830761	✓	✓
DSQ CPG	M830762	30% NH_4OH/EtOH (3:1; v:v)	✓
DSQ PS	M830756 (b) M100501 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
EB380 CPG	M830669	✓	✓
EB380 PS	M830670 (b) M100441 (c)	✓	✓
EB380-MGB PS	M830740 (b) M100431 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
EDQ CPG	M830386	✓	✓
EDQ PS	M830486 (b) M100447 (c)	✓	✓
EDQ-MGB CPG (500 Å)	M830394	30% NH_4OH/EtOH (3:1; v:v)	✓
EDQ-MGB CPG (1000 Å)	M830394-1000	30% NH_4OH/EtOH (3:1; v:v)	✓
EDQ-MGB PS	M830385 (b) M130008 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
FAM-HEG CPG	M830719	✓	✓
FAM-HEG PS	M830718 (b) M100256 (c)	✓	✓
FAM-MGB PS	M830693 (b) M100185 (c)	30% NH_4OH/EtOH (3:1; v:v)	✓
Fmoc-aminohexanediol CPG	M830765	✓	✓
Fmoc-aminohexanediol PS	M830766 (b) M100511 (c)	✓	✓
Fmoc-HEG-MGB PS	M830798 (b) M100508 (c)	✓	✓
Fmoc-hydroxyprolinol CPG	M830768	✓	✓
Fmoc-hydroxyprolinol PS	M830770 (b) M100510 (c)	✓	✓
Gig Harbor Green CPG	M830771	✓	✓
Gig Harbor Green PS	M400090 (b) M100433 (c)	✓	✓

Product name	Product number bulk (b) columns (c)	Recommended deprotection conditions	
		30% NH_4OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C	$^tBuNH_2$:MeOH:H_2O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C
LQ-380 CPG	M830799	✓	✓
LQ-380 PS	M830793 (b) M100505 (c)	✓	✓
LQ-400 CPG	M830800	✓ Partial dye degradation	✓
LQ-400 PS	M830794 (b) M100507 (c)	✓ Partial dye degradation	✓
PBI_3 MGB CPG	M830796	✓	✓
PBI_3 MGB PS	M830797 (b) M100243 (c)	✓	✓
Q-470 CPG	M830562	✓	✓
Q-470 PS	M830568 (b) M100446 (c)	✓	✓
Q-575 CPG	M830563	✓ Partial dye degradation	✓
Q-575 PS	M830569 (b) M100448 (c)	✓ Partial dye degradation	✓
Q-630 CPG	M830564	Not recommended	✓
Q-630 PS	M830570 (b) M100449 (c)	Not recommended	✓
Redmond Red CPG	M830390	✓	✓
Redmond Red PS	M830729 (b) M100445 (c)	✓	✓
Yakima Yellow CPG	M830395	✓	Not recommended
Yakima Yellow PS	M400063 (b) M100443 (c)	✓	Not recommended

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PRODUCT INDEX

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Patents

The Minor groove binder-, dye- and modified base- reagents and their applications are covered by one or more of U.S. Patents Nos.: US6,972,339, US7,112,684, US7,348,146, US7,381,818, US7,541,454, US7,553,643, US7,582,739; US7,601,851, US7,662,942, US7,671,218, US7,767,834, US7,851,606, US7,897,736, US8,008,522, US8,067,177; US8,163,910, US7,205,105, US8,389,745, US6,949,367, US8,465,921, US7,045,610, US7,368,549, US7,485,442, US6,683,173, US7,751,982, US7,715,989, US6,660,845, US6,972,328, US6,962,991, US7,759,126, US9,085,800, US9,056,887, US9,169,256, US9,328,384; US9,334,495, US9,932,643, US9,677,142 US9,988,670, US10,127,349, US10,266,903, US10,590,474, US 677,728, US10,738,346, US10,890,529, US10,975,423, US11,155,713, US11,242,554, US11,320,376, Foreign Patents Nos.: EP3592757A2, EP2971106B1, EP3230468B1, EP3230468B1, EP2714939B1, EP2801626B1, CA2835283C, JP6196968B2, EP2547769B1, EP2997161B1, EP2689031B1, EP2736916B1, JP6182529B2, EP2689031B1, EP1781675B1, JP5214967B2, JP4510626B2, JP4942484B2, EP1687609B1, EP1789587B1, EP01781675B1, JP05214967B2, EP1430147B1, JP4510626B2, EP1687609B1, JP4942484B2, EP1789587B1 , JP4510626B2, EP3668884B1, EP3592757A2 as well as US and international applications that are currently pending.

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