Reagents for Labeling and Modifying Oligonucleotides

Introduction

As the use of synthetic oligonucleotides in biomedical research gets more sophisticated, the simple modification of oligonucleotides becomes an urgent need. Although the automated oligonucleotide synthesis rapidly advances, there are still challenges in meeting the needs for increasingly larger quantities of modified oligonucleotides for therapeutic applications; better high-throughput methods for the screening and PCR markets; and improved synthesis quality of dye modified oligonucleotides for the diagnostic industry.

In recent years, dye-labeled oligonucleotides have received great attentions due to their important biological applications. For certain applications, such as DNA sequencing and *in situ* hybridization (e.g. FISH), oligonucleotides are usually required to be singly labeled. Subsequent detection and analysis relies on the fluorescent properties of the dye, most of which emit light in the visible spectrum. On the other hand, there are some types of biological applications, e.g. probes for real-time PCR quantification of DNA and RNA and allele discrimination (Molecular Beacons™), which require that oligonucleotides be doubly labeled. In the molecules of doubly labeled oligonucleotides one dye acts as a fluorophore, the other as a quencher. When dual-labeled probes are inactive, the light emission from the fluorophore must remain undetected and is absorbed by the quencher dye via a process of so called Fluorescent Resonance Energy Transfer (FRET). Because FRET is a distance-dependent interaction between the excited state of the donor and acceptor dye molecules, their eventual separation in the detection event allows the fluorescence to be detected.

To maximize the FRET efficiency, the FRET pairs need to be carefully selected based on the consideration of quite a few factors such as fluorescence lifetime, the spectral overlap of donor emission with acceptor excitation. AAT Bioquest offers the most comprehensive product line of FRET building blocks for labeling oligonucleotides, including both the classic dyes and our outstanding Tide Fluor™ (donors) and Tide Quenchers™ (acceptors).

5'-Labeling of Oligonucleotides Using Dye Phosphoramidites

Because conventional automated synthesis proceeds from 3' to 5', the 5'-terminus is clearly a good choice for modifying oligonucleotides. As reported in a number of publications, the ability to attach a suitable molecule to the 5'-terminus for use as a label plays a critical role in the continuing development of non-radioactive probes and in DNA sequencing and amplification. A general approach to the modification of the 5'-terminus is to use reagents that would couple to the 5'-hydroxyl of an oligonucleotide.

Dye phosphoramidite reagents have been readily adapted for use in automated synthesizers with little or no modification to existing protocols. These reagents are well compatible with automated DNA synthesizers. In general, dye-labeled oligonucleotides can be deprotected at room temperature in concentrated ammonium hydroxide for a minimum of 24 hours, or shorter time that is appropriated for the protecting groups on the monomers being used. FAM, Dabcyl and Tide Quencher™ (TQ)-labeled oligos can be heated to 55 °C in ammonium hydroxide for extended periods of time. However, TET, TF-3 and Cy-3 labeled oligos are less stable and survive only for a few hours at 55 °C. HEX, TF-5 and Cy-5 labeled oligonucleotides must be deprotected at room temperature and the residual ammonia should be removed immediately after deprotection.

Fluorescein Labeling: AAT Bioquest offers fluorescein-based phosphoramidites that contains no 4, 4'-dimethoxytrityl (DMT) group and can be added only once at the 5'-terminus, thereby terminating synthesis. These fluorescein products include FAM, TET and HEX. FAM phosphoramidite is designed to produce the same fluorescein-type structure as previously prepared using fluorescein isothiocyanate (FITC). The TET and HEX phosphoramidites are designed to take advantage of the multicolor detection capability of modern DNA sequencers and genetic analyzers. For stronger fluorescence intensity, pH-insensitivity and high photostability, we highly recommend that you try our Tide Fluor™ 1 (TF-1) which has the spectral properties that are essentially identical to those of fluorescein.

Rhodamine Labeling: The light-absorbing properties of TAMRA, and spectral overlap with several commonly used fluorophores, including FAM, HEX, TET and JOE, make it useful as a quencher for the dual-

labeled oligo probes. However, its intrinsic fluorescence contributes to the background signal, potentially reducing the sensitivity of assays based on TAMRA. Despite these limitations, TAMRA has been used extensively in the design of probe-based assays, perhaps most notably for Real-Time PCR.

Oligonucleotides can be labeled with TAMRA using two distinct methodologies. Under standard deprotection conditions, TAMRA is not sufficiently stable. It degrades in the presence of ammonium hydroxide. If standard deprotection is required, the oligonucleotide is normally synthesized with an amino group at either the 3'-, or 5'-end and labeled with TAMRA post-synthetically using TAMRA, SE (see below). Oligonucleotides synthesized using UltraMILD monomers can also be labeled directly with TAMRA at the 3'-end using 3'-TAMRA CPG support. Subsequent deprotection of the oligo using potassium carbonate in methanol adequately removes the base protecting groups.

Tide Fluor™ Labeling: TF dyes are optimized to maximize FRET performance through enhancing donor fluorescence intensity. Although EDANS, FAM, TAMRA, ROX, Cy 3 and Cy5 have been widely used to develop a variety of FRET probes, there are still a few limitations for using these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS have severely limited its sensitivity for developing nucleic acid detection probes. Compared to EDANS, fluorescein-based probes (such as FAM, HEX, JOE and TET) have stronger absorption and fluorescence. However the fluorescence of fluorescein-based probes is strongly dependent on pH. They only exhibit the strongest fluorescence at higher pH. This pH dependence makes the fluorescein-based fluorescent probes inconvenient for the assays that require low pH. In addition, most of fluorescein-based probes have quite low photostability, which limits their applications in fluorescence imaging. Among cyanine dyes, non-sulfonated Cy3 and Cy5 are widely used for developing a variety of nucleic acid probes, but they have quite low fluorescence quantum yield in aqueous media. The sulfonated Cy3 and Cy5 have improved fluorescence quantum yield than those of non-sulfonate cyanines. However, the sulfonated Cy3 and Cy5 are more difficult to use in the synthesis of fluorescent oligonucleotides due to the lack of the corresponding sulfonated cyanine phosphoramidites, and are quite cost-prohibitive.

To address these limitations, we have developed Tide Fluor™ donor dyes that are optimized as building blocks for developing FRET oligonucleotides and peptides for a variety of biological applications. Our Tide Fluor™ dyes (such as TF1, TF2, TF3, TF4 and TF5) have stronger fluorescence and higher photostability than the typical fluorophores such as fluoresceins, rhodamines and cyanines as described above. Our TF2 has essentially the same excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily available for the biological applications done with fluoresceins, but has an enhanced performance with our TF2 probes. TF2 has much stronger fluorescence at physiological conditions, and it is much more photostable than FAM probes. Moreover, compared to other fluorescent dyes alternative to fluoresceins and Cy dyes (such as Alexa Fluor™ and Cy3, Cy5 and Cy7), Tide Fluor™ dyes are much more cost-effective with comparable or even better performance for some biological applications.

Key Features of Tide Fluor™ Donors

- **Optimized to pair with Tide Quencher™ dark acceptors to maximize the FRET potentials**
- **Stronger fluorescence intensity to enhance assay sensitivity**
- **pH-insensitive and environment-insensitive fluorescence to simplify assays**
- **Higher photostability to improve the quality of fluorescence imaging**
- **A variety of reactive forms available for conjugations**

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Table 1. Tide Fluor™ building blocks for developing FRET probes

Tide Fluor™ Donor	Excitation	Emission	Major Application Feature*
Tide Fluor™ 1 (TF1)	353 nm	442 nm	TF1 is designed to be a superior fluorophore alternative to EDANS, having the following features: • Much stronger absorption; • Much stronger fluorescence intensity; • Much less environment-sensitive fluorescence.
Tide Fluor™ 2 (TF2)	498 nm	520 nm	TF2 is designed to be a superior fluorophore alternative to fluoresceins (FAM and FITC), having the following features: • Much stronger fluorescence intensity at pH 7; • Much less pH-sensitive fluorescence; • Much more photostable.
Tide Fluor™ 3 (TF3)	557 nm	570 nm	TF3 is designed to be a superior fluorophore alternative to Cy3, having the following features: • Stronger fluorescence intensity; • Much more photostable.
Tide Fluor™ 4 (TF4)	571 nm	596 nm	TF4 is designed to be a superior fluorophore alternative to ROX and Texas Red®, having the following features: • Stronger fluorescence intensity; • Higher conjugation yield; • Longer shelf life.
Tide Fluor™ 5 (TF5)	650 nm	670 nm	TF5 is designed to be a superior fluorophore alternative to Cy5, having the following features: • Stronger fluorescence intensity; • Much more photostable.

*Texas Red® is the trademark of Molecular Probes, Inc.

Tide Quencher™ Acceptor Dyes*:* TQ dyes are optimized to maximize FRET performance through enhancing quenching efficiency. Although DABCYL has been used to develop a variety of FRET applications, its low quenching efficiency of longer wavelength dyes (such as fluoresceins, rhodamines and cyanines) have limited its use in the development of sensitive fluorogenic FRET probes. Additionally, the absorption spectrum of DABCYL is environment-sensitive. AAT Bioquest has developed the robust Tide Quencher™ acceptor dyes for the development of longer wavelength FRET probes. These Tide Quencher™ dark FRET acceptors (such as TQ1, TQ2, TQ3 and TQ4) are optimized to pair with our Tide Fluor™ dyes and the classic fluorophores (such as AMCA, EDANS, FAM, TAMRA, HEX, JOE, TET, ROX, Cy3, Cy5 and Cy7). Like our Tide Fluor™ donors dyes, our Tide Quencher™ acceptor dyes are much more cost-effective with comparable or even better performance for your desired biological applications than other similar products on the market.

We offer a variety of reactive forms for both our Tide Fluor™ donors and Tide Quencher™ acceptors. For in-synthesis labeling of oligonucleotides, we offer both phosphoramidites of our Tide Fluor™ and Tide Quencher™ dyes and their CPG supports. For post labeling of oligonucleotides, we offer both amino-reactive and thiol-reactive Tide Fluor™ and Tide Quencher™ dyes that are water-soluble. Our Tide Quencher™ dyes have been used for developing a variety of Molecular Beacon oligonucleotide probes. Tide Quencher™ dyes are great choice for you to eliminate the limitations of classic quenchers. Tide Quencher ™ dyes are excellent dark quenchers that are individually optimized to pair with all of the popular fluorescent dyes such as fluoresceins and rhodamines. Our Tide Quencher™ series of nonfluorescent dyes cover the full visible spectrum with unusually high efficiency. TQ2 has absorption maximum perfectly matching the emission of FAM while TQ3 is proven to be the best quencher for Cy3. In summary, our Tide Quencher™ dyes have the following advantages:

- *Most Powerful:* enable you to explore the FRET potentials that might be impossible with other quenchers.
- *Versatile Reactive Forms:* convenient for self-constructing your desired FRET biomolecules.
- *A Complete Set of Dyes:* perfectly match your desired fluorescent donors.
- *Enhanced Value:* competitive price with the best performance.

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Table 2. Tide Quencher™ building blocks for developing FRET probes

Tide Quencher™ Acceptor	Absorption	Major Application Features*
Tide Quencher™ 1 (TQ1)	~490 nm	TQ1 is designed to be a superior quencher alternative to DABCYL, having the following features: • Much stronger absorption; • Much higher quenching efficiency; • Versatile reactive forms with desired solubility.
Tide Quencher™ 2 (TQ2)	~520 nm	TQ2 is designed to be a superior quencher to FAM, HEX, TET, JOE, TF2 and rhodamine 6G, having the following features: • Much stronger absorption; • Higher quenching efficiency; • Versatile reactive forms with desired solubility.
Tide Quencher™ 3 (TQ3)	~570 nm	TQ3 is designed to be a superior quencher to TAMRA, TF3 and Cy3, having the following features: • Much stronger absorption; • Higher quenching efficiency; • Versatile reactive forms with desired solubility.
Tide Quencher™ 4 (TQ4)	~610 nm	TQ4 is designed to be a superior quencher to ROX, TF4 and Texas Red®, having the following features: • Much stronger absorption; • Higher quenching efficiency; • Versatile reactive forms with desired solubility.
Tide Quencher™ 5 (TQ5)	~670 nm	TQ5 is designed to be a superior quencher to Cy5, TF5 and Cy5.5, having the following features: • Much stronger absorption; • Higher quenching efficiency; • Versatile reactive forms with desired solubility.

*Texas Red® is the trademark of Molecular Probes, Inc.

3’-Labeling of Oligonucleotides Using Dye CPG Supports

Besides the dye CE phosphoramidites described above, AAT Bioquest also offers dye CPG supports. Dye CPG supports have traditionally been used to add the dye labels at the 3’-terminus. Dye CPGs are used to introduce a dye molecule to the 3'-terminus of oligonucleotides. Our dye CPGs are derived from dye carboxylic acids and are attached via an amide linkage, giving an oligo product that is much easier to be purified by HPLC. The use of dye CPGs in oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with some necessary modifications. Different dye CPGs might require different cleavage methods. The cleavage of the oligonucleotides from the FAM and Tide Quenchers™ (TQs) supports is similar to the standard ammonium hydroxide cleavage. TAMRA CPG has to be deprotected under very mild conditions to safeguard the base-labile TAMRA fluorophore. We recommend the use of UltraMild monomers and the use of potassium carbonate in methanol for deprotection. An alternative procedure using t-butylamine/methanol/water (1:1:2) might allow the use of regular monomers.

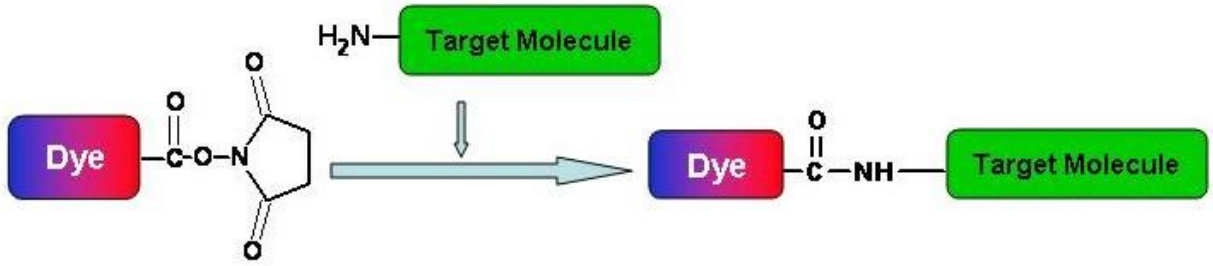
Indirect Modification of Oligonucleotides

AAT Bioquest currently offers 5'-amino-modifiers (Cat. # 4300 and Cat. # 4304). These reagents are designed for use in automated synthesizers to functionalize the 5'-terminus of a target oligonucleotide with a primary amine moiety. The resulted amino-modified oligonucleotides can be conjugated to a variety of tag molecules such as fluorophores, biotins, alkaline phosphatase and HRP. Due to the increased possibility of side reactions during the deprotection of modified oligonucleotides, it is recommended that the ammonium hydroxide treatment be carried out at a lower temperature than that used for unmodified oligonucleotides. The MMT protecting group of the 5'-amino-

modifier (Cat. # 4300) can be removed on the synthesizer by deblocking until the yellow color elutes totally. The solution of MMT cation produced by acid deprotection is yellow and is not well quantified by trityl monitors. The modified oligonucleotide may be purified using a Poly-Pak cartridge, HPLC or gel electrophoresis. Poly-Pak cartridge purification is accomplished using the trityl-on procedure. HPLC may be performed either before or after the attachment of the label. If purification is desired prior to the label attachment, the MMT group should not be removed from the oligonucleotide as the lipophilic character of the MMT group aids in HPLC purification.

AAT Bioquest also offers Chemical Phosphorylation Reagent (CPR, Cat. # 6001). CPR has proved to be fast and convenient for chemical phosphorylation of the 5'-terminus of oligonucleotides. In addition, this reagent has proved its utility for simple phosphorylation of the 3'-terminus. It is introduced as the first addition to any nucleoside support, followed by normal synthesis of the target oligonucleotide. After the standard ammonium hydroxide deprotection, the linkage decomposes and is ß-eliminated from the target molecule, leaving a phosphate group at the 3'-terminus. The final DMT group may be removed on the synthesizer or it may be retained to aid in purification. If the DMT group is retained, it may be removed on a purification cartridge or, by treating the oligonucleotide with acetic acid:water (80:20) at room temperature for 1 hour following purification.

Post-Labeling of Oligonucleotides Using Dye Succinimidyl Esters



Succinimidyl esters are proven to be the best reagents for labeling amine-modified oligos because the amide bonds formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. AAT Bioquest offers highly purified classic fluorescent dye succinimidyl esters and our outstanding Tide Fluor™ (TF) and Tide Quencher™ (TQ) succinimidyl esters. Our dye succinimidyl esters are packed under nitrogen to enhance their shelf life, and also packed in different sizes to provide you the maximum convenience in handling these moisture-sensitive reagents.

There are a few factors that need to be considered when SE compounds are used for conjugation reactions:

- *Solvents:* For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- *Reaction pH:* The labeling reactions of aliphatic amine-containing oligonucleotides with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the amine groups of oligos. Thus amine acylation reactions are usually carried out above pH 7.5. Oligo modifications by succinimidyl esters can typically be done at pH ranging from 7.5 to 8.5, whereas isothiocyanates may require a pH 9.0-10.0 for optimal conjugations.
- *Reaction Buffers:* Buffers that contain free amines such as Tris and glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) must also be removed (such as via dialysis) before performing dye conjugations.
- *Reaction Temperature:* Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

Why should you use our Tide Quencher™ dyes (TQ1, TQ2, TQ3, TQ4 and TQ5)?

- ***Most Powerful:*** TQs enable you to explore the maximum FRET potentials with oligonucleotides;
- ***Versatile Reactive Forms:*** TQs are convenient for self-constructing your desired FRET biomolecules;
- ***A Complete Set of Dyes:*** TQs perfectly match any fluorescent donors that you select;
- ***Enhanced Value:*** We offer you the most competitive price with the best performance.

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Table 1. Tide Fluor™ and Tide Quencher™ FRET building blocks from AAT Bioquest

CAT. #	PRODUCT NAME	UNIT
2238	Tide Fluor™ 1 acid [TF1 acid] *Superior replacement to EDANS*	100 mg
2239	Tide Fluor™ 1 amine [TF1 amine] *Superior replacement to EDANS*	5 mg
2240	Tide Fluor™ 1 CPG [TF1 CPG] *500 Å* *Superior replacement to EDANS*	100 mg
2241	Tide Fluor™ 1 CPG [TF2 CPG] *1000 Å* *Superior replacement to EDANS*	100 mg
2242	Tide Fluor™ 1 maleimide [TF1 maleimide] *Superior replacement to EDANS*	5 mg
2300	Tide Fluor™ 1 phosphoramidite [TF1 CEP] *Superior replacement to EDANS*	100 umoles
2243	Tide Fluor™ 1 phosphoramidite [TF1 CEP] *Superior replacement to EDANS*	1 g
2244	Tide Fluor™ 1, succinimidyl ester [TF1 SE]*Superior replacement to EDANS*	5 mg
2245	Tide Fluor™ 2 acid [TF2 acid] *Superior replacement to fluorescein*	25 mg
2246	Tide Fluor™ 2 amine [TF2 amine] *Superior replacement to fluorescein*	1 mg
2249	Tide Fluor™ 2 CPG [TF2 CPG] *500 Å* *Superior replacement to fluorescein*	1 g
2250	Tide Fluor™ 2 CPG [TF2 CPG] *1000 Å* *Superior replacement to fluorescein*	1 g
2247	Tide Fluor™ 2 maleimide [TF2 maleimide] *Superior replacement to fluorescein*	1 mg
2301	Tide Fluor™ 2 phosphoramidite [TF2 CEP] *Superior replacement to fluorescein*	100 umoles
2251	Tide Fluor™ 2 phosphoramidite [TF2 CEP] *Superior replacement to fluorescein*	50 umoles
2248	Tide Fluor™ 2, succinimidyl ester [TF2 SE]*Superior replacement to fluorescein*	5 mg
2268	Tide Fluor™ 3 acid [TF3 acid] *Superior replacement to Cy3*	25 mg
2269	Tide Fluor™ 3 amine [TF3 amine] *Superior replacement to Cy3*	1 mg
2272	Tide Fluor™ 3 CPG [TF3 CPG] *500 Å* *Superior replacement to Cy3*	1 g
2273	Tide Fluor™ 3 CPG [TF3 CPG] *1000 Å* *Superior replacement to Cy3*	1 g
2270	Tide Fluor™ 3 maleimide [TF3 maleimide] *Superior replacement to Cy3*	1 mg
2302	Tide Fluor™ 3 phosphoramidite [TF3 CEP] *Superior replacement to Cy3*	100 umoles
2274	Tide Fluor™ 3 phosphoramidite [TF3 CEP] *Superior replacement to Cy3*	50 umoles
2271	Tide Fluor™ 3, succinimidyl ester [TF3 SE]*Superior replacement to Cy3*	5 mg
2285	Tide Fluor™ 4 acid [TF4 acid] *Superior replacement to ROX & Texas Red*	10 mg
2286	Tide Fluor™ 4 amine [TF4 amine] *Superior replacement to ROX & Texas Red*	1 mg
2287	Tide Fluor™ 4 maleimide [TF4 maleimide] *Superior replacement to ROX & Texas Red*	1 mg
2303	Tide Fluor™ 4 phosphoramidite [TF4 CEP] *Superior replacement to ROX & Texas Red*	100 umoles
2288	Tide Fluor™ 4 phosphoramidite [TF4 CEP] *Superior replacement to ROX & Texas Red*	1 g
2289	Tide Fluor™ 4, succinimidyl ester [TF4 SE]*Superior replacement to ROX & Texas Red*	5 mg
2278	Tide Fluor™ 5 acid [TF5 acid] *Superior replacement to Cy5*	10 mg
2279	Tide Fluor™ 5 amine [TF5 amine] *Superior replacement to Cy5*	1 mg
2282	Tide Fluor™ 5 CPG [TF5 CPG] *500 Å* *Superior replacement to Cy5*	1 g
2283	Tide Fluor™ 5 CPG [TF5 CPG] *1000 Å* *Superior replacement to Cy5*	1 g
2280	Tide Fluor™ 5 maleimide [TF5 maleimide] *Superior replacement to Cy5*	5 mg
2304	Tide Fluor™ 5 phosphoramidite [TF1 CEP] *Superior replacement to Cy5*	100 umoles
2284	Tide Fluor™ 5 phosphoramidite [TF5 CEP] *Superior replacement to Cy5*	100 umoles
2281	Tide Fluor™ 5, succinimidyl ester [TF5 SE]*Superior replacement to Cy5*	5 mg
2190	Tide Quencher™ 1 acid [TQ1 acid]	100 mg
2192	Tide Quencher™ 1 amine [TQ1 amine]	5 mg
2193	Tide Quencher™ 1 CPG [TQ1 CPG] *500 Å*	100 mg
2194	Tide Quencher™ 1 CPG [TQ1 CPG] *1000 Å*	100 mg
2196	Tide Quencher™ 1 maleimide [TQ1 maleimide]	5 mg

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2100	Tide Quencher™ 1 phosphoramidite [TQ1 phosphoramidite]	100 umoles
2198	Tide Quencher™ 1 phosphoramidite [TQ1 phosphoramidite]	100 umoles
2199	Tide Quencher™ 1 succinimidyl ester [TQ1 SE]	25 mg
2200	Tide Quencher™ 2 acid [TQ2 acid]	100 mg
2202	Tide Quencher™ 2 amine [TQ2 amine]	5 mg
2203	Tide Quencher™ 2 CPG [TQ2 CPG] *500 Å*	100 mg
2204	Tide Quencher™ 2 CPG [TQ2 CPG] *1000 Å*	100 mg
2206	Tide Quencher™ 2 maleimide [TQ2 maleimide]	5 mg
2105	Tide Quencher™ 2 phosphoramidite [TQ2 phosphoramidite]	100 umoles
2208	Tide Quencher™ 2 phosphoramidite [TQ2 phosphoramidite]	100 umoles
2210	Tide Quencher™ 2 succinimidyl ester [TQ2 SE]	25 mg
2220	Tide Quencher™ 3 acid [TQ3 acid]	100 mg
2222	Tide Quencher™ 3 amine [TQ3 amine]	5 mg
2223	Tide Quencher™ 3 CPG [TQ3 CPG] *500 Å*	100 mg
2224	Tide Quencher™ 3 CPG [TQ3 CPG] *1000 Å*	100 mg
2226	Tide Quencher™ 3 maleimide [TQ3 maleimide]	5 mg
2110	Tide Quencher™ 3 phosphoramidite [TQ3 phosphoramidite]	100 umoles
2228	Tide Quencher™ 3 phosphoramidite [TQ3 phosphoramidite]	100 umoles
2230	Tide Quencher™ 3 succinimidyl ester [TQ3 SE]	25 mg
2180	Tide Quencher™ 4 acid [TQ4 acid]	100 mg
2182	Tide Quencher™ 4 amine [TQ4 amine]	25 mg
2184	Tide Quencher™ 4 CPG [TQ4 CPG] *500 Å*	1 g
2186	Tide Quencher™ 4 CPG [TQ4 CPG] *1000 Å*	1 g
2187	Tide Quencher™ 4 maleimide [TQ4 maleimide]	25 mg
2115	Tide Quencher™ 4 phosphoramidite [TQ4 phosphoramidite]	100 mmoles
2188	Tide Quencher™ 4 phosphoramidite [TQ4 phosphoramidite]	1 g
2189	Tide Quencher™ 4 succinimidyl ester [TQ4 SE]	25 mg
2231	Tide Quencher™ 5 acid [TQ5 acid]	100 mg
2232	Tide Quencher™ 5 amine [TQ5 amine]	25 mg
2233	Tide Quencher™ 5 CPG [TQ5 CPG] *500 Å*	1 g
2234	Tide Quencher™ 5 CPG [TQ5 CPG] *1000 Å*	1 g
2235	Tide Quencher™ 5 maleimide [TQ5 maleimide]	25 mg
2120	Tide Quencher™ 5 phosphoramidite [TQ5 phosphoramidite]	100 mmoles
2236	Tide Quencher™ 5 phosphoramidite [TQ5 phosphoramidite]	1 g
2237	Tide Quencher™ 5 succinimidyl ester [TQ5 SE]	25 mg

Table 2. Classic fluorescent labeling reagents for oligonucleotide synthesis from AAT Bioquest

CAT. #	PRODUCT NAME	UNIT
320	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed isomers*	100 mg
321	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed isomers*	1 g
6007	3'-DABCYL CPG *500 Å*	1 g
6008	3'-DABCYL CPG *1000 Å*	1 g
6009	5'-DABCYL C6 phosphoramidite	1 g
611	EDANS acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid]	10 g
615	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	1 g
616	EDANS sodium salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic acid, sodium salt]	10 g
101	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for labeling peptides and oligos*	10 g
102	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for labeling peptides and oligos*	25 g
106	6-FAM [6-Carboxyfluorescein] *Single isomer*	100 mg
107	6-FAM [6-Carboxyfluorescein] *Validated for labeling peptides and oligos*	1 g
108	6-FAM [6-Carboxyfluorescein] *Validated for labeling peptides and oligos*	5 g
6028	5(6)-FAM Phosphoramidite	100 umoles
6029	5(6)-FAM Phosphoramidite	10x100 umoles
6016	6-FAM phosphoramidite [5'-Fluorescein phosphoramidite]	100 umoles
6017	6-FAM phosphoramidite [5'-Fluorescein phosphoramidite]	10x100 umoles
6020	6-FAM phosphoramidite [5'-Fluorescein phosphoramidite]	1 g
112	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides and oligos*	1 g
116	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Single isomer*	10 mg
117	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides and oligos*	100 mg
118	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides and oligos*	1 g
120	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure grade*	100 mg
121	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure grade*	1 g
122	5-FITC [FITC Isomer I; fluorescein-5-isothiocyanate] *UltraPure grade*	10 g
125	6-FITC [FITC Isomer II, fluorescein-6-isothiocyanate] *UltraPure grade*	10 g
6015	FITC phosphoramidite [Fluorescein phosphoramidite]	1 g
6018	6-Fluorescein phosphoramidite	100 umoles
6019	6-Fluorescein phosphoramidite	10x100 umoles
6021	6-TET phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite]	50 umoles
6011	3'-Fluorescein CPG *1000 Å*	1 g
6014	3'-(6-Fluorescein) CPG *1000 Å*	1 g
6010	3'-Fluorescein CPG *500 Å*	1 g
6013	3'-(6-Fluorescein) CPG *500 Å*	1 g
130	Fluorescein-5-maleimide	25 mg
6022	6-HEX phosphoramidite [5'-Hexachlorofluorescein phosphoramidite]	1 g
6024	6-HEX phosphoramidite [5'-Hexachlorofluorescein phosphoramidite]	10x100 umoles
6026	6-HEX phosphoramidite [5'-Hexachlorofluorescein phosphoramidite]	100 umoles
382	6-ROX [6-Carboxy-X-rhodamine] *Single isomer*	25 mg
394	Sunnyvale Red™ SE *Superior 6-ROX Replacement*	5 mg
395	6-ROX Plus™, acid *Enhanced stability*	100 mg

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397	6-ROX Plus™, succinimidyl ester *Enhanced stability*	5 mg
398	6-ROX Plus™, succinimidyl ester *Enhanced stability*	50 mg
210	6-ROX, SE [6-Carboxy-X-rhodamine, succinimidyl ester] *Single isomer*	20x0.25 mg
392	6-ROX, SE [6-Carboxy-X-rhodamine, succinimidyl ester] *Single isomer*	5 mg
480	Sulforhodamine 101 sulfonyl chloride [Texas Red®]*	10 mg
361	5-(and 6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Validated for labeling peptides and oligos*	1 g
362	5-(and 6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Validated for labeling peptides and oligos*	5 g
366	6-TAMRA [6-Carboxytetramethylrhodamine] *Single isomer*	10 mg
367	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling peptides and oligos*	100 mg
368	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling peptides and oligos*	1 g
6051	3'-TAMRA CPG *1000 Å*	1 g
6050	3'-TAMRA CPG *500 Å*	1 g
6053	5'-TAMRA phosphoramidite	100 mg
370	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester] *Mixed isomers*	25 mg
371	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides and oligos*	100 mg
372	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides and oligos*	1 g
376	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester] *Single isomer*	5 mg
377	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides and oligos*	100 mg
378	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides and oligos*	1 g
6021	6-TET phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite]	50 umoles
6025	6-TET phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite]	10x100 umoles
6027	6-TET phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite]	100 umoles

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