Tide Fluor™ and Tide Quencher™ Dyes, Optimized for Maximizing The Power of Fluorescence Resonance Energy Transfer (FRET)

Fluorescence resonance energy transfer (FRET) is the transfer of the excited state energy from the initially excited donor (D) to an acceptor (A). The donor molecules typically emit at shorter wavelengths that overlap with the absorption of acceptors. The process is a distance-dependent interaction between the electronic excited states of two molecules *without emission of a photon*. FRET is the result of long-range dipole-dipole interactions between the donor and acceptor as illustrated in Figure 1. A few excellent recent reviews are listed in the references for further information.

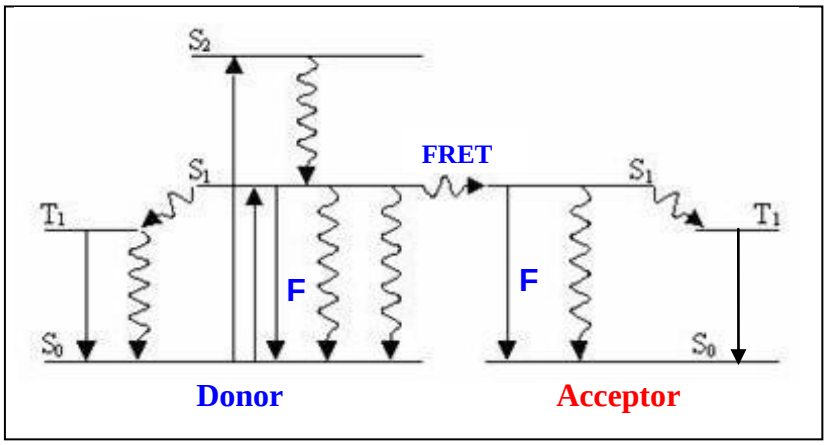

Figure 1 Diagram of FRET process from the donor to acceptor molecule. Horizontal lines represent discrete electron energy levels for each molecule. Energy levels are labeled as either singlet states (S) or triplet states (T) with subscripts numbered zero, one or two (representing the ground state, first excited electronic state or second excited electronic state). A molecule's electrons generally reside in the ground state, S_0. Electrons may be excited to higher energy levels by a number of processes, including light absorption and chemical reaction.

As shown in Figure 1, an excited donor molecule has several routes to release its captured energy returning to the ground state. The excited state energy can be dissipated to the environment (as light or heat) or transferred directly to a second acceptor molecule, sending the acceptor to an excited state. The later process is called FRET.

- *Internal Energy Conversions:* The rapid return of electrons from the second (or higher) excited state to the first excited state is termed internal conversion. Energy is released through rapid solvent relaxation.
- *Light Emissions:* Light is released by either the transition from S_1 to S_0 (fluorescence emission) or transition from T_1 to S_0 (phosphorescence emission).
- *Fluorescence Resonance Energy Transfer:* FRET occurs when donor and acceptor molecules are within a specified range, usually within 10 to 100 Å. In the process of FRET, the excited-state energy of a donor is transferred to an acceptor molecule. Photons of light aren't involved in this transfer. In Figure 1, the pathway leading from the S_1 level of the donor to the S_1 level of the acceptor represents FRET. Once excited, the acceptor can return to the ground state by the same pathways as described for the donor. If the acceptor molecule is also fluorescent, it can emit light at its characteristic wavelength, which is always longer than the emission wavelength of the donor.
- *Collisional Quenchings:* Collisions between an excited-state fluorophore and other molecules will sometimes quench the fluorophore, returning it to the ground state without emission of a photon. Collisional quenchers include molecular oxygen and electron scavengers such as Cu^{2+}, Mn^{2+}, halides, isothiocyanate, nitrite and nitrate ions. Collisional quenching primarily occurs when these ions are present in the *millimolar* range or higher. Therefore, under most experimental conditions, collisional quenching is usually negligible.

Optimal FRET Conditions

The efficiency (E%) and rate (k_T) of FRET are respectively expressed as follows:

$$E\% = k_T/(\tau_D^{-1} + k_T) \quad [1]$$

$$k_T = R_o^6 \gamma^{-6} \tau_D^{-1} \quad [2]$$

Where τ_D is the decay time of the donor in the absence of acceptor; γ is the donor-acceptor (D-A) distance; R_o is the Förster distance where FRET has 50% efficiency (typically in the range of 20-60Å). R_o is determined by the following equation:

$$R_o^6 = 8.79 \times 10^{23} [k^2 n^{-4} \Phi_D J(\lambda)] \quad [3]$$

Where k^2 is dipole-dipole orientation factor (ranging from 0 to 4, $k^2 = 2/3$ for randomly oriented donors and acceptors); n is refractive index [The refractive index is generally known from solvent composition or estimated for macromolecules such as proteins and nucleic acids. n is often assumed to be that of water (n=1.33) for aqueous solutions, or to be that of small organic molecules (n=1.39) for organic solutions]. Φ_D is the fluorescence quantum yield of donor in the absence of acceptor. $J(\lambda)$ is FRET spectral overlap integral as illustrated in Figure 2, and is determined by the following equation:

$$J(\lambda) = \int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d(\lambda) \quad [4]$$

Where $F_D(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda+\Delta\lambda$ with the total intensity (area under the curve) normalized unity; ε_A is extinction coefficient of the acceptor at λ.

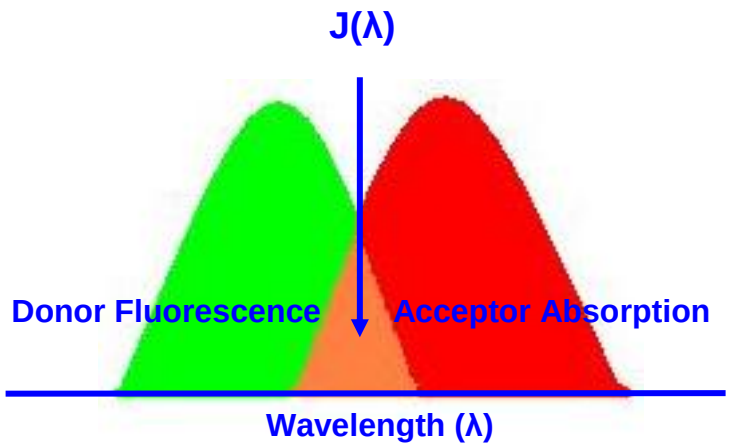

Figure 2. Schematic representation of the FRET spectral overlap integral

From the above equations, it is easily concluded that an efficient FRET should have the following conditions:

- *Distance between Donor and Acceptor:* Donor and acceptor molecules must be in close proximity (typically 10–100 Å).
- *Spectral Overlap:* The absorption spectrum of the acceptor must overlap fluorescence emission spectrum of the donor (see Figure 2).
- *Dipole Orientation:* Donor and acceptor transition dipole orientations must be approximately parallel.

Typical Biological Applications of FRET Probes

As discussed above, the rate and efficiency of FRET are dependent on the inverse sixth power of the intermolecular separation. FRET is highly efficient if the donor and acceptor are positioned within the Förster radius, which is typically 30-60 Å. The distance dependence of FRET has resulted in its widespread biological applications since the distances are comparable with the dimensions of biological macromolecules. For instance, one helical turn in B-DNA spans 33.2 Å. Further, FRET efficiency falls dramatically as the distance between the donor and acceptor exceeds the Förster radius, making FRET an important technique for investigating a variety of biological phenomena in which tracking physical proximity is important. If the donor molecule's fluorescence is quenched, it indicates that the donor and acceptor molecules are within the Förster radius, whereas if the donor fluoresces at its characteristic wavelength, it denotes that the distance between the donor and acceptor molecules has increased beyond the Förster radius. In recent years, FRET-based assays have found broad applications in detecting proteases, real-time assays of hybridization, PCR monitoring and DNA interactions in living cells.

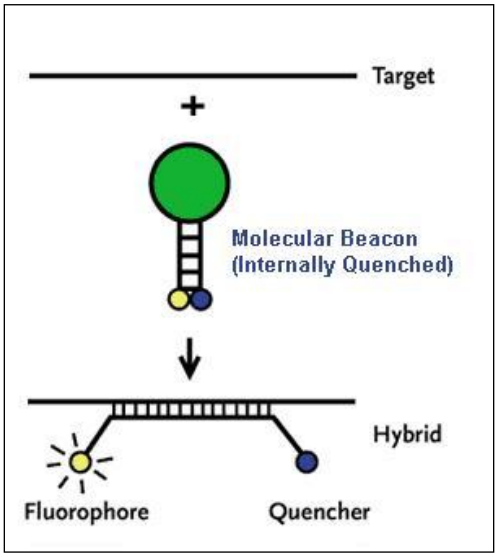

Figure 3. Schematic representation of fluorogenic Molecular Beacon probes

Nucleic Acid Detection: Molecular Beacons, which are 'hairpin' oligonucleotides containing both a fluorescent reporter dye and a quencher as shown in Figure 3, are widely used to detect DNA hybridization. In the absence of target, the fluorescent reporter and quencher molecules are brought close together in the probe's self-complementary stem structure, and the fluorescent signal is suppressed. When the Molecular Beacon hybridizes to its target, the fluorescent reporter and the quencher are separated, and the reporter dye emits at its characteristic wavelength. Molecular Beacons have enhanced specificity for their targets, making them superior in hybridization-based investigations of single nucleotide polymorphisms (SNPs). Molecular Beacons have been used in

hybridization-based assays to identify rifampin-resistant Mycobacterium and to detect virus replication in HIV type 1-infected individuals. Molecular Beacons have also been widely used to detect nucleases. Dual-labeled FRET probes are commonly used to detect the 5' exonuclease activity of Taq polymerase. The two labels on the FRET oligonucleotide can be separated by as many as 25-30 bases. If the oligonucleotide is intact, the donor molecule's signal is quenched. During the course of the assay, the cleavage event separates the donor molecule from its acceptor, restoring the donor's characteristic emission. If a dark quencher is used as the acceptor molecule, multiple FRET probes can be used, each labeled with a unique fluorophore, making these probes amenable to multiplex assays.

AAT Bioquest offers a complete set of dye phosphoramidites and dye CPG supports for preparing FRET oligonucleotides including the classic dyes (such as FAM, HEX, TET and JOE) and superior oligo-labeling dyes such as our Tide Fluor™ and Tide Quencher™ dyes. 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 below. Our TF2 has the essentially same excitation and emission wavelengths as those of carboxyfluoresceins (FAM), making them readily used for the biological applications that were done with fluoresceins, but have enhanced performance with TF2. Compared to FAM probes, TF2 has much stronger fluorescence at physiological conditions, and it is much more photostable. 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 your desired biological applications.

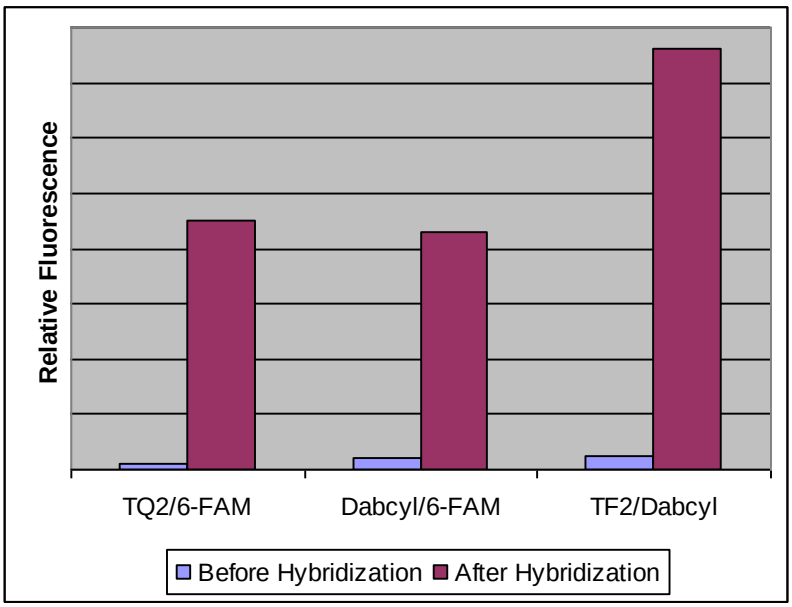

Figure 4. Hybridization-induced fluorescence enhancement of Molecular Beacon oligonucleotide probes that contain Tide Fluor™ dyes as donor or Tide Quencher™ dyes as acceptors.

Besides our outstanding Tide Fluor™ donor dyes, AAT Bioquest has also developed the robust Tide Quencher™ acceptor dyes. 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™ donor 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.

To facility your research, 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.

Protease Detection: Proteases are involved in a number of physiological processes. The detection of proteases and the screening of specific protease inhibitors are essential in the discovery of potential drugs for the

treatment and management of protease-related diseases. A variety of donor and acceptor molecules (either fluorescent or non-fluorescent) are attached to the peptide/protein (forming the internally quenched conjugates), and are used to detect a protease depending on the sequences of the amino acids. The donor and acceptor molecules are carefully chosen so that the absorption of the acceptor overlaps with the fluorescence of the donor, thus ensuring that the fluorescence is quenched through resonance energy transfer. Enzyme hydrolysis of the substrate results in spatial separation of the donor and the acceptor molecules, thereby restoring the donor's fluorescence. For example, EDANS and DABCYL are used to label a peptide that contains the HIV protease cleavage site. Proteolytic cleavage releases a fragment containing only the EDANS fluorophore, thus liberating it from the quenching effect of the nearby DABCYL chromophore. AAT Bioquest offers a variety of reagents for developing FRET-based protease probes and assays. Please visit our websites at www.aatbio.com for protease assays or www.bioconjugation.com for FRET probe building blocks.

HIV protease has been identified as one of the key targets for the development of anti-AIDS drugs. The aspartic HIV protease, a 10-12 kD of human immunodeficiency virus-1 (HIV-1), is required for the post-translational cleavage of the precursor polyproteins, Pr*gag* and Pr*gag-pol*. These cleavages are essential for the maturation of HIV infectious particles. A Tide Fluor™ 2/Tide Quencher™ 2-derived FRET peptide has been conveniently used in our Amplite Flurorimetric HIV Protease Assay Kit for high throughput screening of HIV-1 protease inhibitors and continuous quantification of HIV-1 protease activity. The sequence of this FRET peptide is derived from the native p17/p24 cleavage site on Pr_{gag} for HIV-1 protease. In the FRET peptide, the fluorescence of Tide Fluor™ 2 is quenched by Tide Quencher™ 2 until this peptide is cleaved into two separate fragments by HIV-1 protease.

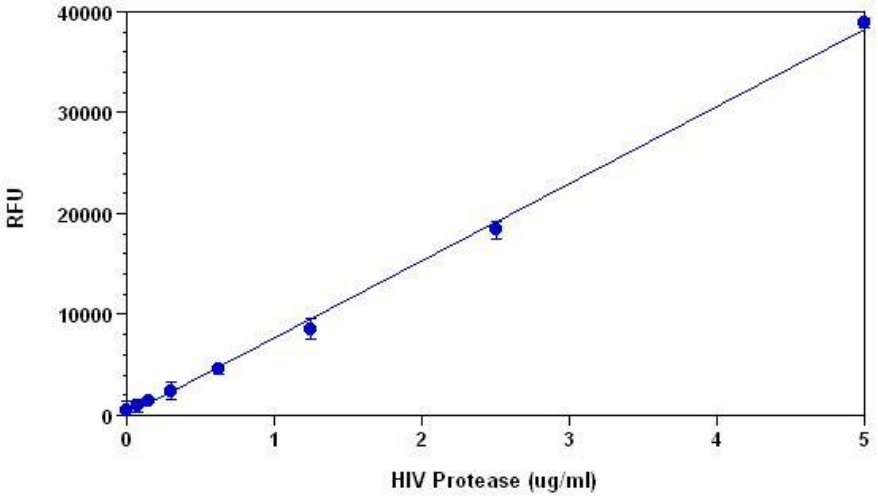

Figure 5. HIV Protease cleavage of Tide Fluor™ 2/Tide Quencher™ 2-derived FRET peptide. Upon cleavage, the fluorescence of Tide Fluor™ 2 is recovered, and can be monitored at EX/EM = 490/520 nm. With excellent fluorescence quantum yield and longer excitation and emission wavelength, the fluorescence signal of Tide Fluor™ 2 is less interfered by the autofluorescence of cell components and test compounds, thus providing better assay sensitivity.

Tide Fluor™ Donor Dyes, 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 some limitations in the use of these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS has severely limited its sensitivity for developing protease assays and 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 difficult to use in the synthesis of fluorescent oligonucleotides, and are quite cost-prohibitive.

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**

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 below. Our TF2 has the essentially same excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily used for the biological applications that were done with fluoresceins, but have enhanced performance with our TF2 probes. Compared to FAM probes, TF2 has much stronger fluorescence at physiological conditions, and it is much more photostable. 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 your desired biological applications.

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

Tide Fluor™ Donor	Excitation	Emission	Major Application Features*
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)	554 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)	652 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, 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) has 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.

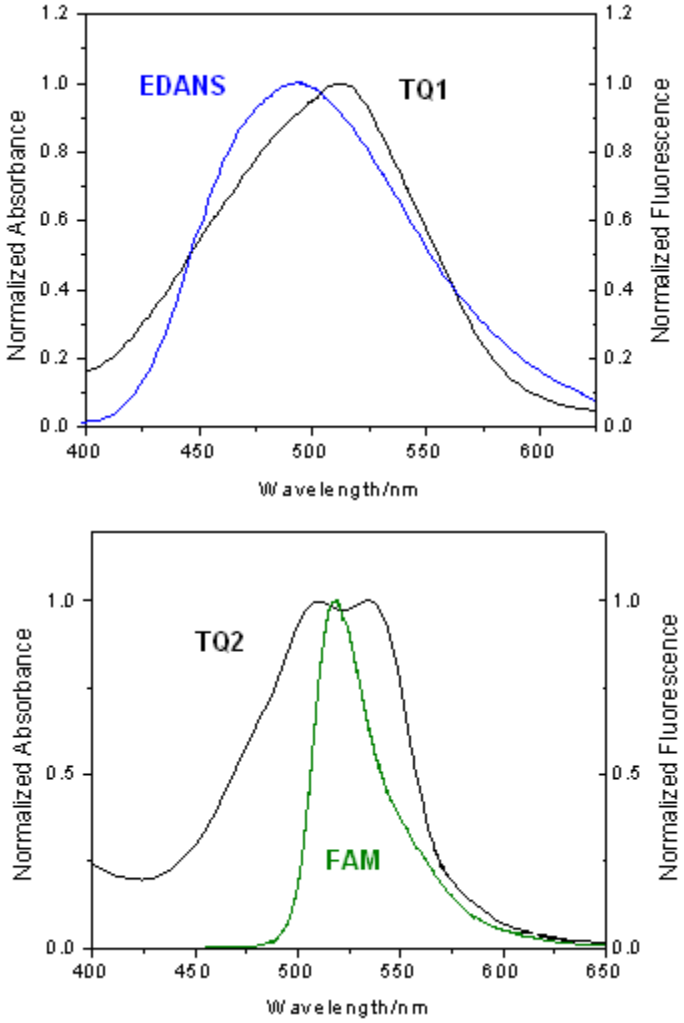

Figure 6. The spectral overlap of Tide Quencher™ 1 (TQ1, top) and Tide Quencher™ 2 (TQ2, bottom) with EDANS (top) and FAM (bottom).

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.

Besides their broad applications in the development of Molecular Beacon probes, our Tide Quencher™ dyes have also been used to develop various protease substrates such as HIV protease (see above), MMPs and secretases. In some cases, it has demonstrated greatly improved enzyme performance. This may be partly due to the red-shifted absorption spectrum that overlaps better with the emission spectrum of fluoresceins, rhodamines and cyanines. 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 (See Figure 6) while TQ3 is proven to be the best quencher for TAMRA and 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.

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.

Selection of FRET Donors and Acceptors

Probes incorporating fluorescent donor/non-fluorescent acceptor (*e.g.* Dabcyl) combinations have been developed primarily for the detection of proteolysis and nucleic acid hybridization. This principle has also been used to develop other FRET-based assays such as receptor/ligand interactions, distribution and transport of lipids, membrane potential sensing and cyclic AMP detection.

The donor and acceptor molecules can be the same or different. In most applications, they are different dyes. FRET can be detected either by the appearance of sensitized fluorescence of the acceptor or by the intensity ratio change of donor/acceptor (if the acceptor is fluorescent), or the fluorescence decrease of the donor. In the later

case, acceptor can be either fluorescent or non-fluorescent. A number of R_o values for various D/A pairs have been published. Larger R_o values of FRET pairs give higher FRET efficiency. Fluorescein/tetramethylrhodamine pairs have been most widely used to develop FRET-based assays. EDANS/DABCYL pair has been intensively used to develop FRET-based nucleic acid probes and protease substrates. MCA (7-methoxycoumarin-4-acetic acid) and DNP (2, 4-dinitroaniline) are another pair of donor/acceptor for developing FRET-based fluorescent probes. Compared with the pair of EDANS/DABCYL, MCA/DNP pair usually has shorter and weaker wavelength of fluorescence signal. However, the pair often demonstrates better affinity or turnover rate due to their smaller sizes. DNP is also a good FRET acceptor paired with tryptophan (Trp), 2-aminobenzoic acid (Abz) or Abz derivatives such as Abz(*N*-Me).

Table 3. Some typical R_o values of D/A pairs*

Donor	**Acceptor**	**R_o (Å)**
Fluorescein	Tetramethylrhodamine	49-56
IAEDANS **	FITC	49
IAEDANS	5-(Iodoacetamido)fluorescein	49
Fluorescein	Fluorescein	44
EDANS	DABCYL	33
Tryptophan	IAEDANS	22
Tryptophan	Dansyl	21-24
Tryptophan	Pyrene	28
Dansyl	Fluorescein	33-41
Naphthalene	Dansyl	22
Pyrene	Coumarin	39
B-Phycoerythrin	Cy5	79

* The value may change under different conditions; **IAEDANS = 5-((Iodoacetyl)amino)naphthalene-sulfonic acid.

Table 4 summarizes our Tide Quencher™ FRET building blocks designed for you to develop FRET probes for the demanding applications. Based on our in-house research and experience, it is recommended to use the FRET pairs marked in green. The pairs marked in cyan color are OK to use, but less efficient than the ones marked in green. We have proved that these recommended FRET pairs have demonstrated high sensitivity and low background in our protease and nucleic acid detection assays. There are also other factors that you need to consider besides the FRET efficiency, such as pH, multiplexing and buffer interference, etc.

Table 4. Recommended FRET pairs for developing Molecular Beacon nucleic acid detection and protease assays

	FRET Dark Quenchers (Acceptors)					
FRET Fluorophores (Donors)	**Dabcyl**	**TQ1**	**TQ2**	**TQ3**	**TQ4**	**TQ5**
EDANS	+ + + + +	+ + + + +	+ + +			
MCA	+ + + + +	+ + + + +	+ + +			
TF1	+ + + + +	+ + + + +	+ + +			
FAM/FITC	+ + +	+ + +	+ + + + +	+ + +		
TF2	+ + +	+ + +	+ + + + +	+ + +		
Cy2/HEX/TET/JOE/Rhdoamine 6G			+ + +	+ + + + +	+ + +	
Cy3/TAMRA			+ + +	+ + + + +	+ + +	
TF3			+ + +	+ + + + +	+ + +	
ROX/Texas Red®				+ + +	+ + + + +	+ + +
TF4				+ + +	+ + + + +	+ + +
Cy5/Cy5.5					+ + +	+ + + + +
TF5					+ + +	+ + + + +

Table Legends: + + + + + Best to use; + + + OK to use; Not recommended to use.

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Table 1. Tide Fluor™ and Tide Quencher™ FRET building blocks

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*	1 g
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 g
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 m g
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*	1 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]	25 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

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 umoles
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 umoles
2236	Tide Quencher™ 5 phosphoramidite [TQ5 phosphoramidite]	1 g
2237	Tide Quencher™ 5 succinimidyl ester [TQ5 SE]	25 mg

Table 2. Classic FRET building blocks

CAT. #	PRODUCT NAME	UNIT
56	AFC [7-Amino-4-trifluoromethylcoumarin] *Fluorescence reference standard*	1 g
57	AFC [7-Amino-4-trifluoromethylcoumarin] *Validated for labeling peptides*	5 g
58	AFC [7-Amino-4-trifluoromethylcoumarin] *Validated for labeling peptides*	25 g
51	AMC [7-Amino-4-methylcoumarin] *Fluorescence reference standard*	1 g
52	AMC [7-Amino-4-methylcoumarin] *Validated for labeling peptides*	5 g
53	AMC [7-Amino-4-methylcoumarin] *Validated for labeling peptides*	25 g
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
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
103	5-FAM [5-Carboxyfluorescein] *Single isomer*	100 mg
104	5-FAM [5-Carboxyfluorescein] *Validated for labeling peptides*	1 g
105	5-FAM [5-Carboxyfluorescein] *Validated for labeling peptides*	5 g
106	6-FAM [6-Carboxyfluorescein] *Single isomer*	100 mg
107	6-FAM [6-Carboxyfluorescein] *Validated for labeling oligos*	1 g
108	6-FAM [6-Carboxyfluorescein] *Validated for labeling oligos*	5 g
127	5(6)-FAM cadaverine	100 mg
128	5-FAM cadaverine	100 mg
112	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides and oligos*	1 g
113	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester] *Single isomer*	10 mg
114	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides*	100 mg
115	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester] *Validated for labeling peptides*	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 oligos*	100 mg
118	6-FAM, SE [6-Carboxyfluorescein, succinimidyl ester] *Validated for labeling 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
130	Fluorescein-5-maleimide	25 mg
5001	FMOC-Asp(EDANS)-OH	1 g
5002	FMOC-Asp(EDANS)-OH	5 g
5003	FMOC-Asp(5/6-FAM)-OH	100 mg
5004	FMOC-Asp(5-FAM)-OH	100 mg
5005	FMOC-Asp(5/6-TAMRA)-OH	100 mg
5006	FMOC-Asp(5-TAMRA)-OH	100 mg
5010	FMOC-Glu(EDANS)-OH	100 mg
5011	FMOC-Glu(EDANS)-OH	5 g
5012	FMOC-Glu(5/6-FAM)-OH	100 mg

5013	FMOC-Glu(5-FAM)-OH	100 mg
5014	FMOC-Glu(5/6-TAMRA)-OH	100 mg
5015	FMOC-Glu(5-TAMRA)-OH	100 mg
5042	FMOC-Lys(5/6-FAM)-OH	1 g
5043	FMOC-Lys(5-FAM)-OH	100 mg
5044	FMOC-Lys(5/6-TAMRA)-OH	100 mg
5045	FMOC-Lys(5-TAMRA)-OH	100 mg
550	7-Hydroxycoumarin-3-carboxylic acid	250 mg
551	7-Hydroxycoumarin-3-carboxylic acid, succinimidyl ester	50 mg
560	7-Methoxycoumarin-3-carboxylic acid	1 g
561	7-Methoxycoumarin-3-carboxylic acid	5 g
563	7-Methoxycoumarin-3-carboxylic acid, succinimidyl ester	100 mg
86	Rhodamine 110	1 g
480	Sulforhodamine 101 sulfonyl chloride [Texas Red®]*	10 mg
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
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*	20 x 0.25 mg
392	6-ROX, SE [6-Carboxy-X-rhodamine, succinimidyl ester] *Single isomer*	5 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
363	5-TAMRA [5-Carboxytetramethylrhodamine] *Single isomer*	10 mg
364	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for labeling peptides*	100 mg
365	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for labeling peptides*	1 g
366	6-TAMRA [6-Carboxytetramethylrhodamine] *Single isomer*	10 mg
367	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling oligos*	100 mg
368	6-TAMRA [6-Carboxytetramethylrhodamine] *Validated for labeling oligos*	1 g
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
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester] *Single isomer*	5 mg
374	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides*	100 mg
375	5-TAMRA, SE [5-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling peptides*	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 oligos*	100 mg
378	6-TAMRA, SE [6-Carboxytetramethylrhodamine, succinimidyl ester] *Validated for labeling oligos*	1 g