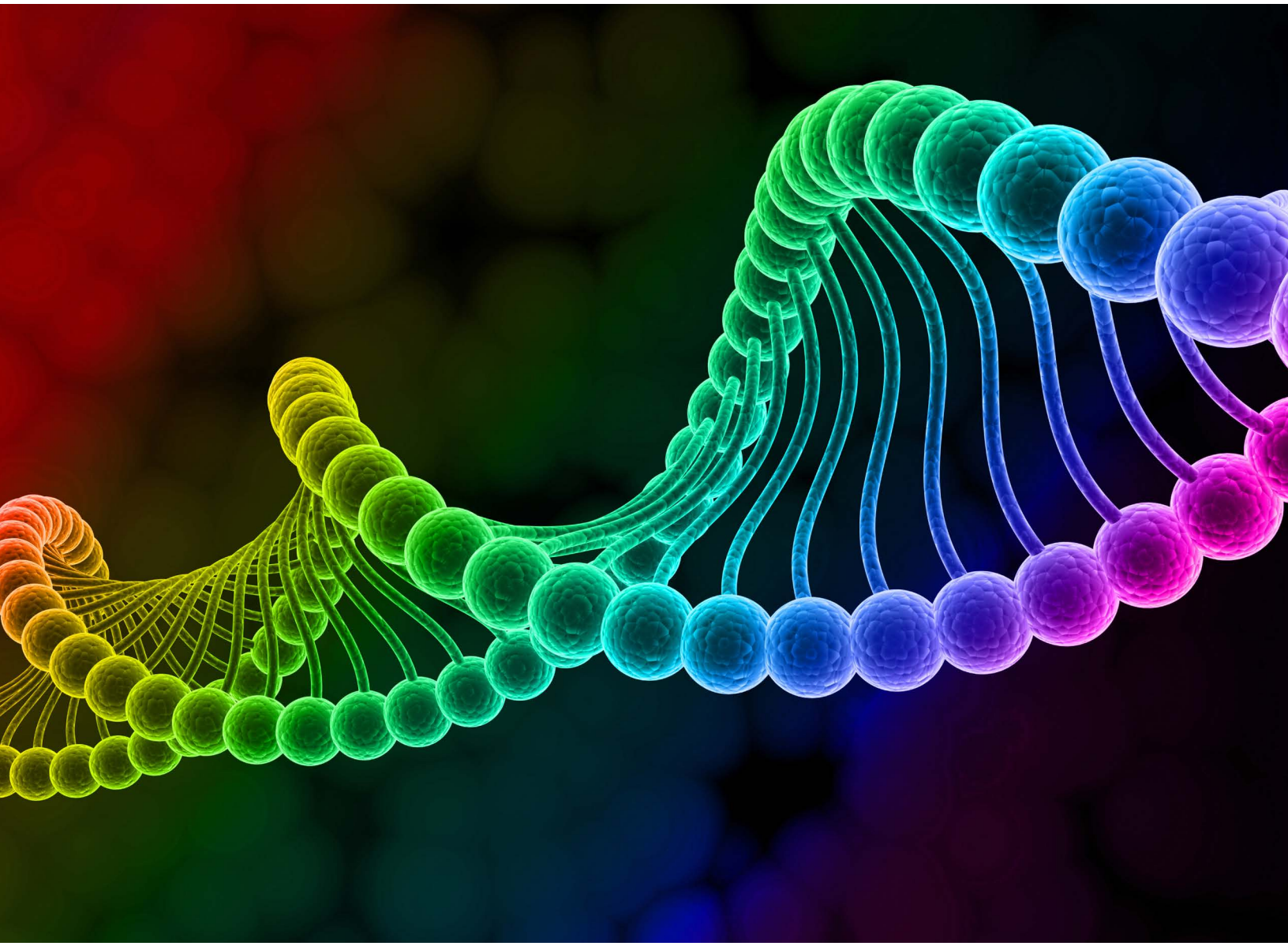
# Oligonucleotide Labeling Reagents



Dye CPGs | Dye Phosphoramidites | Reactive Dyes

Stratech

AAT Bioquest®

## *Our Mission*

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

## *Our Story*

AAT Bioquest®, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

# Table of Contents

## Base Symbols

A: Adenine
C: Cytosine
G: Guanine
T: Thymine
U: Uracil

## Abbreviations Used in This Catalog

Ab: Absorption
CF: Correction factor
DMF: Dimethylformamide
DMSO: Dimethylsulfoxide
EC: Extinction coefficient
Em: Emission
Ex: Excitation
FACS: Fluorescence-associated cell sorting
FAM: Carboxyfluorescein
FITC: Fluorescein isothiocyanate
FRET: Fluorescence resonance energy transfer
NHS: N-Hydroxysuccinimide
PCR: Polymerase chain reaction
ROX: X-rhodamine
SC: Sulfonyl chloride
SE: Succinimidyl ester
TAMRA: Carboxytetramethylrhodamine
TF: Tide Fluor™
TRF: Time resolved fluorescence
TQ: Tide Quencher™
TR: Texas Red®

## Trademarks of AAT Bioquest

AAT Bioquest®
California Red™
Helix Fluor™
iFluor™
SunRed™
Tide Fluor™
Tide Quencher™

## Trademarks of Other Companies

Alexa Fluor® (Invitrogen)
BHQ® (Biosearch Technologies)
Cy2®, Cy3®, Cy5®, Cy5.5® and Cy7® (GE Healthcare)
DyLight™ (ThermoFisher)
IRDye® (LI-COR)
QSY® (Life Technologies)
QXL™ (AnaSpec)
Texas Red® (Invitrogen)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**International Distributors:**
**See Back Cover**

## TERMS AND CONDITIONS OF SALE

**1. Prices, Orders and Changes:** Prices shown are in US currency. Please call us for current prices if you require this information prior to placing your order. We guarantee our written quotations for 60 days. You may not cancel purchase orders unless such cancellation is expressly agreed by us. In such event, you will be advised of the total charge for such cancellation. You agree to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on us by our suppliers, and any other cost resulting from cancellation of this order.

**2. Delivery:** In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

**3. Payment:** Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

**4. Warranties:** The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

**5. Returns:** We must authorize any returns. We will not accept return shipments unless we have given prior written permission and shipping instructions. Goods may not be returned for credit except with our permission, and then only in strict compliance with our return shipment instructions. Any returned items may be subject to a 20% restocking fee. In many cases, items ordered in error cannot be returned because of the sensitive nature of many of our products and the difficulty and expense of requalifying returned items. If items are accepted for return, they must be in new, unopened, unused and undamaged condition, and you will be charged a per-unit 20% restocking charge.

**6. Use of Our Products:** Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

**7. Patent Disclaimer:** We do not warrant that the use or sale of our products will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

**8. Miscellaneous:** We reserve the right to discontinue our products or change specifications or prices of our products and to correct any errors or omissions at any time without incurring obligations.

# Custom Products and Services



## Our Technologies

***Amplite™*** enzyme-based detection platform is optimized for measuring horseradish peroxidase (HRP), alkaline phosphates, luciferase, beta-galactosidase, lactamase, oxidase, protein kinases, protein phosphatases, phosphodiesterases, proteases, cytochrome P450, histone deacetylase (HDAC) and cell signaling molecules such as NAD/NADH, NADP/NADPH, $IP_3$, cAMP and cGMP etc.

***Cell Explorer™*** cell labeling platform is a complete set of tools for tracking live cells. This platform is also widely used for sorting mixed populations of cells.

***Cell Navigator™*** cell staining platform is a complete set of tools for selective labeling subcellular structures of live, fixed and dead cells.

***Cell Meter™*** cellular functional assay platform is a complete set of tools for functional analysis of cellular events and real time-monitoring of cell functions.

***iFluor™*** superior fluorescent labeling dyes are optimized for labeling proteins and nucleic acids. This group of dyes span from UV to infrared wavelength with good photostability and brightness.

***mFluor™*** superior fluorescent labeling dyes are optimized for flow cytometry applications.

***PhosphoWorks™*** detection platform is a set of tools for detection of ATP, ADP, AMP, phosphate, pyrophosphate, phosphoproteins and phosphopeptides.

***Quest View™*** colorimetric protease platform is a sensitive and robust tool for rapid detection of protease and glycosidase biomarkers. This technology platform has been licensed by a few diagnostic companies for developing rapid diagnostic tests.

***RatioWorks™*** superior cellular dyes are a sensitive and robust tool set for ratio imaging and real time monitoring of cellular functions (such as pH and ions) in live cells.

***Screen Quest™*** assay kits are a set of HTS-ready tools for high throughput screening of biochemical and cellular targets such as protein kinases, proteases, HDAC, cell apoptosis and cytotoxicity, GPCR, ion channels, ADME and transporters.

***Tide Fluor™ and Tide Quencher™*** superior labeling dyes are specially optimized for labeling nucleotides and peptides. This platform offers the best value in the industry. It is second to none in terms of performance and cost. This technology platform has been licensed by a few diagnostic companies for developing IVD diagnostic tests.

***trFluor™*** superior fluorescent labeling dyes are optimized for developing time-resolved fluorescence-based assays. It has been used for developing HTS assay technologies for many drug discovery targets.

## Our Services

Besides the catalog products we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays, custom bioconjugation and custom high throughput screening of drug discovery targets.

### Custom Assay Design and Development

At AAT Bioquest we not only make probes and assay kits, but also use them extensively ourselves. Scientists at AAT Bioquest are experts on assay design and have developed a wide variety of tests that range from biochemical detection to cellular functions. Our assay options include:

- Enzyme activities
- Binding assays
- Cell-based assays
- Microplate assays
- Flow cytometric analysis
- Fluorescence imaging

### Custom Conjugation

AAT Bioquest offers the best and the most rapid bioconjugation service in the industry.

- Biotinylation
- Fluorescence labeling (iFluor™, mFluor™, APC, RPE and PerCP)
- Enzyme labeling (AP and HRP)
- Small molecule conjugation

### Custom Screening

AAT Bioquest offers on-demand high-throughput screening and pharmacology profiling assays with multiple methodologies. Functional assays are designed, validated and customized to the needs of our pharmaceutical and biotechnology industry clients. These assays are aimed at assessing and monitoring the efficacy, tolerability and safety parameters of candidate compounds for treating and/or diagnosing cancer, infectious disease, autoimmunity and transplantation. Our screening options include:

- Full assay development for a target of your choice
- Optimization of your assay protocol for HTS
- Multiple assay platforms and detection methods
- Custom data analysis

### Custom Synthesis of Fluorophores and Luminophores

AAT Bioquest is recognized by the top pharmaceutical companies and diagnostic companies as a key provider of novel fluorescent dyes and luminescent probes. Over the years we have developed and synthesized many enabling fluorescent and luminescent probes for running a variety of challenging biological detection tasks.

# Fluorescent Dyes for Labeling Oligonucleotides

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Dye-labeled oligonucleotides are important tools in biochemical and cellular studies. Fluorescent oligonucleotides have been extensively used in fluorescence fluorimetry, fluorescence microscopy, fluorescence polarization spectroscopy, time-resolved fluorescence (TRF) and fluorescence resonance energy transfer (FRET). FRET oligonucleotides are widely used for diagnosing infectious diseases based on the molecular beacon and other technologies. FRET oligonucleotides have been also used for cell analysis via fluorescence-associated cell sorting (FACS) either *in vivo* or *in vitro* for research and diagnostic purposes. The most important characteristics of fluorescent oligonucleotides are high sensitivity and non-radioactive detection.

## 2.1 Selection of a Fluorescent Dye for Labeling Oligonucleotides

A fluorescent dye can be attached to an oligonucleotide at a specific point through a covalent bond depending on the sequence of oligonucleotide. The linkage between dye and oligonucleotide is a covalent bond, which is stable and not destructive under most biological conditions. In some cases, a functional linker is introduced between dye and oligonucleotide to minimize the alteration of oligonucleotide biological activity. For all the oligonucleotide labelings, the dye need be attached at a defined position: 3′, 5′, or in the middle of sequence. In general, the preferred fluorescent labels should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules. AAT Bioquest offers a variety of fluorescent labeling dyes for facilitating the conjugation of dyes to oligonucleotides that are used for biological studies. These fluorescent dyes include coumarins, fluoresceins, rhodamines and cyanines. Among them, our Tide Fluor™, California Red™, SunRed™ and Helix Fluor™ dyes are optimized for labeling oligonucleotides.

## 2.2 Coumarin Dyes

Coumarin dyes are predominantly used for preparing blue fluorescent oligonucleotides. Compared to other fluorophores, coumarin dyes generally are much smaller molecules, thus have less interference on the biological activities of the molecules to be labeled. Among coumarins, AMCA, 7-hydroxy, 7-alkoxy and 7-aminocoumarin are more frequently used. As one of the brightest blue fluorophores, AMCA might be the best blue fluorescent dye for labeling oligonucleotides due to its high fluorescence quantum yield and pH-insensitivity.

Our Tide Fluor™ 1 (TF1) is a water-soluble derivative of AMCA, and can be used for improving oligonucleotide water solubility if the labeling of an oligonucleotide by AMCA significantly decreases its water solubility. AMCA and TF1 have almost identical spectral properties. Besides AMCA and TF1, two of the most frequently used blue fluorescent labeling dyes, AAT Bioquest also offers a variety of coumarin dyes for labeling peptides, oligonucleotides and other biomolecules.

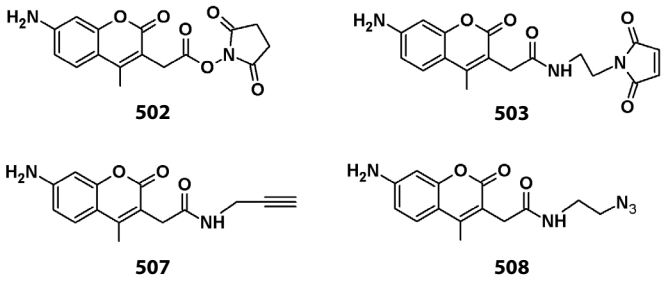


**Figure 2.1.** The chemical structures of typical coumarin dyes for labeling oligos.

**Table 2.1 Coumarin Dyes for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 507 | AMCA Alkyne | 1 mg | 353 | 455 | 19,000 | 0.183 |
| 508 | AMCA Azide | 1 mg | 353 | 455 | 19,000 | 0.183 |
| 503 | AMCA C2 Maleimide | 5 mg | 353 | 455 | 19,000 | 0.183 |
| 504 | AMCA Ethylenediamine | 5 mg | 353 | 455 | 19,000 | 0.183 |
| 502 | AMCA, Succinimidyl Ester | 10 mg | 353 | 455 | 19,000 | 0.183 |
| 506 | DEAC, SE [7-Diethylaminocoumarin-3-carboxylic Acid, Succinimidyl Ester] | 25 mg | 427 | 478 | 40,000 | Not Determined |
| 556 | 7-Hydroxy-4-methylcoumarin-3-acetic Acid, Succinimidyl Ester | 25 mg | 364 | 458 | 25,000 | 0.168 |
| 551 | 7-Hydroxycoumarin-3-carboxylic Acid, Succinimidyl Ester | 50 mg | 419 | 447 | 35,000 | 0.226 |
| 553 | 7-Hydroxycoumarin-4-acetic Acid, Succinimidyl Ester | 25 mg | 360 | 450 | 18,000 | Not Determined |
| 558 | MCA, Succinimidyl Ester [7-Methoxycoumarin-4-acetic Acid, Succinimidyl Ester] | 25 mg | 322 | 390 | 15,000 | 0.134 |
| 563 | 7-Methoxycoumarin-3-carboxylic Acid, Succinimidyl Ester | 100 mg | 358 | 410 | 25,000 | 0.071 |


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## 2.3 Fluorescein Dyes

Fluorescein dyes are the most common fluorescent derivatization reagents for covalently labeling oligonucleotides. In addition to their high absorptivity, excellent fluorescence quantum yields and good water solubility, fluorescein dyes have an excitation maximum (494 nm) that closely matches the 488 nm spectral line of the argon-ion laser, making them important fluorophores for fluorescence microscopy and flow cytometry applications. 6-FAM derivatives have been predominantly used for labeling oligonucleotides. AAT Bioquest offers the most complete product line of FAM for labeling oligonucleotides.

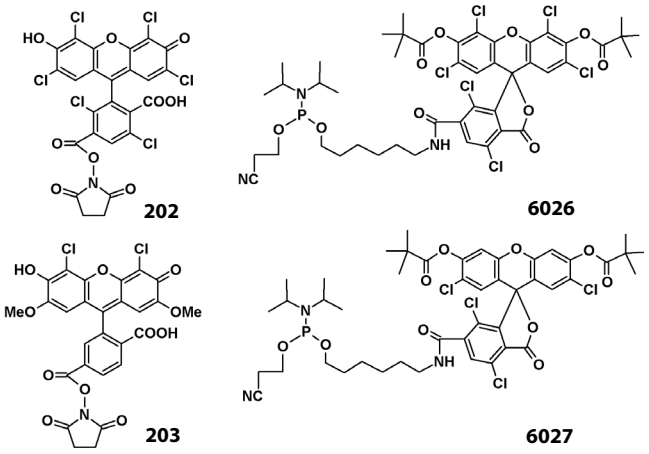


**Figure 2.2.** The chemical structures of typical fluorescein dyes for labeling oligos.

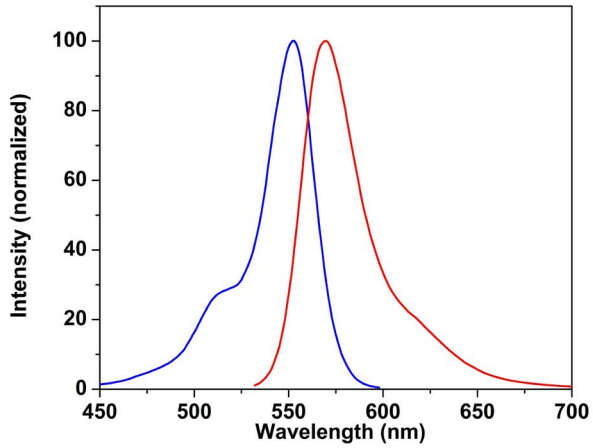


**Figure 2.3** The excitation and emission of Helix Fluor™ 575, SE (Cat# 251).

TET, HEX, JOE, VIC and NED fluorescein derivatives are widely used for the multicolor genetic detections by taking advantage of the excellent capability of modern DNA sequencers and genetic analyzers. Among them, TET, HEX and JOE are offered by quite a few commercial vendors (including AAT Bioquest), making them readily available for genetic research and development. However, there are very few commercial vendors that carry NED and VIC dyes. Upon the repeated requests from our customers, we have developed Helix Fluor™ 575 (Cat# 251) and Helix Fluor™ 555 (Cat# 250) as the excellent replacements for NED and VIC dyes. They closely match the spectral properties of NED and VIC respectively. Both Helix Fluor™ 575 (Cat# 251) and Helix Fluor™ 555 (Cat# 250) are succinimidyl esters that readily react with amino-modified oligonucleotides.

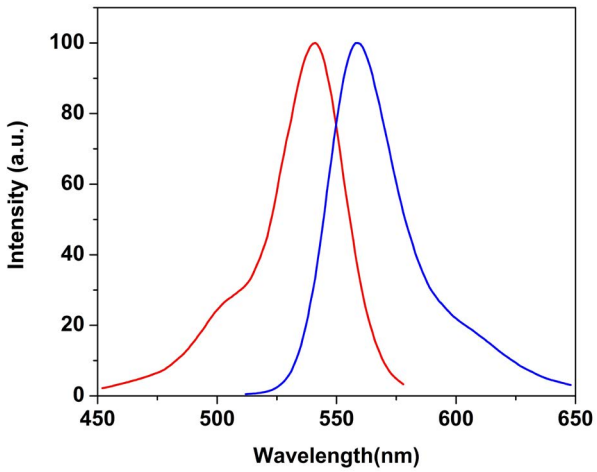


**Figure 2.4** The excitation and emission of Helix Fluor™ 555, SE (Cat# 250) .

6-JOE, a xanthene dye, refers to 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein. 6-JOE fluoresces in the yellow region of the visible spectrum and can be effectively quenched by BHQ®-1 or our TQ2 dye. 6-JOE succinimidyl ester is the major commercial product used for labeling oligonucleotides. The 6-JOE modification incorporates the 6-JOE moiety at either 5' terminus or thymidine of an oligonucleotide. The 6-JOE modification conjugated to a modified thymidine may participate in hybridization. 6-JOE modified oligonucleotides can be used in a wide array of applications, including dual-labeled fluorogenic probes for real-time PCR.

Compared to other fluorescein succinimidyl esters, 6-JOE SE generally gives much lower yield, and the resulted conjugates are also more difficult to be purified. Recently we have analyzed a number of commercial 6-JOE SE materials, and found that all the commercial 6-JOE SE materials contain a few impurities which often complicate the conjugations of 6-JOE SE with amino-modified oligonucleotides as shown in Figure 2.5. The impurities associated with the commercial 6-JOE SE materials include unreactive free 6-JOE acid, 3,6-JOE disuccinimidyl ester and 3-JOE succinimidyl ester. In particular, the conjugate resulted from 3-JOE succinimidyl ester is difficult to be separated from the desired 6-JOE conjugate. 6-JOE SE offered by AAT Bioquest is essentially free of 3,6-JOE disuccinimidyl ester and 3- JOE succinimidyl ester as shown in Figure 2.6.

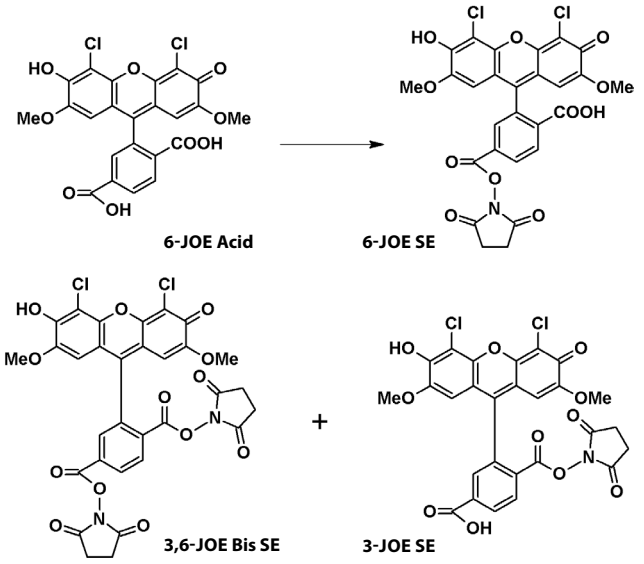


**Figure 2.5.** The reactions of 6-JOE, SE with amino-modified oligonucleotides are complicated by the impurities resulted from the manufacturing process of 6-JOE, SE.

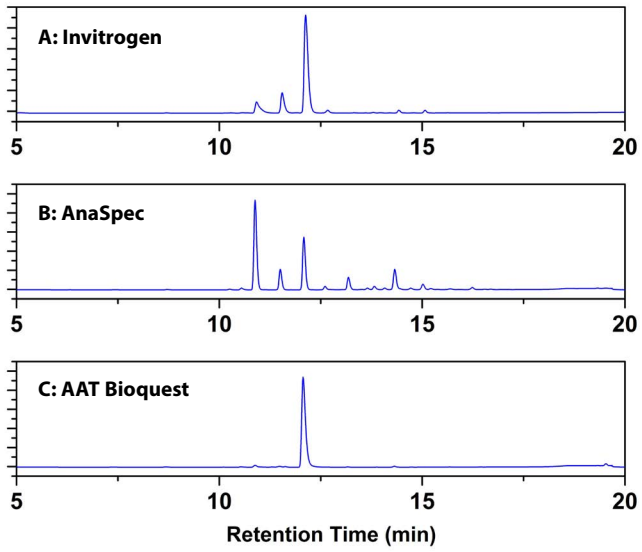


**Figure 2.6.** HPLC analysis of 6-JOE, SE materials from different commercial vendors. Vendor A: Invitrogen (Lot#: 1454485); Vendor B: AnaSpec (Lot#: 96700-1); Vendor C: AAT Bioquest (Lot#: 099133).

### Table 2.2 Fluorescein Dyes for Labeling Oligonucleotides

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1} M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 134 | 6-FAM Alkyne | 10 mg | 494 | 521 | 75,000 | 0.255 |
| 133 | 6-FAM Azide | 10 mg | 494 | 521 | 75,000 | 0.255 |
| 6016 | 6-FAM Phosphoramidite [5'-Fluorescein phosphoramidite] | 100 µmoles | 494 | 521 | 75,000 | 0.255 |
| 116 | 6-FAM, SE [6-Carboxyfluorescein, Succinimidyl Ester] *Single Isomer* | 10 mg | 494 | 521 | 75,000 | 0.255 |
| 6045 | Helix Fluor™ 6-JOE Phosphoramidite | 50 µmoles | 520 | 548 | 73,000 | 0.325 |
| 250 | Helix Fluor™ 555, Succinimidyl Ester | 1 mg | 542 | 558 | 76,000 | 0.354 |
| 251 | Helix Fluor™ 575, Succinimidyl Ester | 1 mg | 546 | 575 | 85,000 | 0.354 |
| 241 | 6-HEX Alkyne | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 240 | 6-HEX Azide | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 6026 | 6-HEX Phosphoramidite [5'-Hexachlorofluorescein Phosphoramidite] | 100 µmoles | 533 | 550 | 74,000 | 0.300 |
| 202 | 6-HEX, SE [6-Carboxy-2',4,4',5',7,7'-hexachlorofluorescein, Succinimidyl Ester] | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 249 | 6-JOE Alkyne | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 248 | 6-JOE Azide | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 203 | 6-JOE, SE [6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, Succinimidyl Ester] | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 245 | 6-TET Alkyne | 5 mg | 521 | 536 | 76,000 | 0.191 |
| 244 | 6-TET Azide | 5 mg | 521 | 536 | 76,000 | 0.191 |
| 6027 | 6-TET Phosphoramidite [5'-Tetrachlorofluorescein Phosphoramidite] | 100 µmoles | 521 | 536 | 76,000 | 0.191 |
| 211 | 6-TET, SE [6-Carboxy-2',4,7',7-tetrachlorofluorescein, Succinimidyl Ester] | 5 mg | 521 | 536 | 78,000 | 0.191 |


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## 2.4 Rhodamine Dyes

Rhodamine dyes are supplements to fluoresceins as they offer longer wavelength emission maxima and provide opportunities for multicolor labeling and staining. Rhodamines exhibit higher photostability than fluoresceins and coumarins. Carboxytetramethylrhodamine (TAMRA) is a common fluorophore for preparing oligonucleotide conjugates. Sulforhodamine 101 sulfonayl chloride (Texas Red®) is another popular oligonucleotide labeling dye. However, Texas Red® is unstable and gives much lower coupling yield than other carboxyrhodamine dyes (such as 5-TAMRA, SE).

AAT Bioquest has recently developed California Red™, a replacement superior to Texas Red®. California Red™ has the spectral properties almost identical to those of Texas Red® and similar water solubility. However California Red™ is much more stable and gives much higher conjugation yield than Texas Red® (See Table 2.4 on page 10).

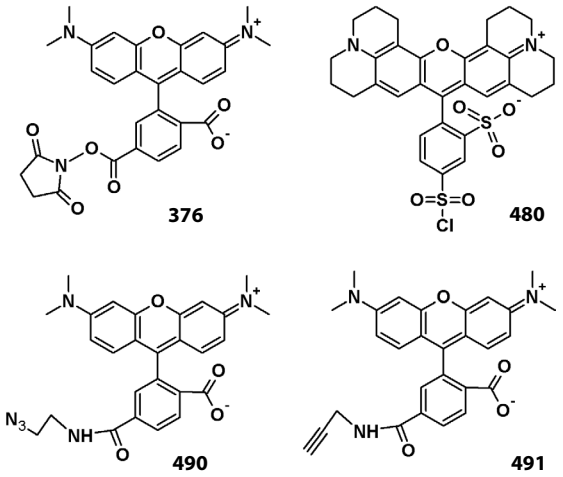


**Figure 2.7.** The chemical structures of typical rhodamine dyes for labeling oligos.



### Table 2.3 Rhodamine Dyes for Labeling Oligonucleotides

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1} M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 479 | California Red™, SE | 1 mg | 583 | 603 | 100,000 | 0.456 |
| 473 | California Red™, SE | 5 mg | 583 | 603 | 100,000 | 0.456 |
| 470 | Lissamine Rhodamine B Sulfonyl Chloride [Sulforhodamine B sulfonyl chloride] | 100 mg | 568 | 583 | 85,000 | 0.288 |
| 380 | 5(6)-ROX [5-(and 6)-Carboxy-X-rhodamine] *Mixed Isomers* | 100 mg | 568 | 591 | 85,000 | 0.301 |
| 390 | 5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, Succinimidyl Ester] | 25 mg | 576 | 601 | 93,000 | 0.301 |
| 495 | 6-ROX Alkyne | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 494 | 6-ROX Azide | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 426 | 6-ROX C2 Maleimide | 1 mg | 575 | 602 | 95,000 | 0.307 |
| 392 | 6-ROX, SE [6-Carboxy-X-rhodamine, Succinimidyl Ester] *Single Isomer* | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 480 | Sulforhodamine 101 Sulfonyl Chloride [Also known as Texas Red®] | 10 mg | 588 | 601 | 100,000 | 0.456 |
| 370 | 5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] | 25 mg | 546 | 575 | 78,000 | 0.320 |
| 423 | 5(6)-TAMRA C6 Maleimide | 5 mg | 544 | 575 | 75,000 | 0.320 |
| 491 | 6-TAMRA Alkyne | 5 mg | 547 | 573 | 75,000 | 0.335 |
| 490 | 6-TAMRA Azide | 5 mg | 547 | 573 | 75,000 | 0.335 |
| 425 | 6-TAMRA C6 Maleimide | 5 mg | 544 | 575 | 75,000 | 0.335 |
| 357 | 6-TAMRA Cadaverine | 5 mg | 544 | 575 | 75,000 | 0.335 |
| 6051 | 6-TAMRA CPG *1000 Å* | 1 g | 546 | 578 | 75,000 | 0.335 |
| 359 | 6-TAMRA Ethylenediamine | 5 mg | 544 | 575 | 75,000 | 0.335 |
| 419 | 6-TAMRA Maleimide [Tetramethylrhodamine-6-maleimide] *Single Isomer* | 1 mg | 540 | 567 | 75,000 | 0.335 |
| 376 | 6-TAMRA, SE [6-Carboxytetramethylrhodamine, Succinimidyl Ester] | 5 mg | 547 | 573 | 78,000 | 0.335 |
| 485 | Texas Red® Alkyne *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.456 |
| 484 | Texas Red® Azide *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.456 |

## 2.5 California Red™ and SunRed™, Superior Replacements for Texas Red® and Texas Red®-X for Labeling Oligonucleotides

Although sulforhodamine 101 acid chloride (also called Texas Red®) is the most popular labeling reagent among sulfonyl chlorides, it is quite unstable in water, especially at the higher pH required for reactions with aliphatic amines. Texas Red® reacts with both aliphatic amines and aromatic amines indiscriminately. In addition, the labeling efficiency of Texas Red® is extremely low compared to dye succinimidyl esters. California Red™ SE is a succinimidyl ester. It is an excellent replacement for Texas Red®. California Red™ reacts with amine compounds such as amino acids, oligonucleotides and proteins to give bright red fluorescent conjugates that are extremely stable. Compared to Texas Red®, California Red™ has much higher labeling efficiency, and more importantly, the resulted conjugates are more fluorescent than the corresponding Texas Red® conjugates for long oligonucleotides. The conjugates of California Red™ have the identical excitation and emission wavelengths to those of Texas Red®. Our in-house studies indicated that California Red™ is more stable than Texas Red® under the same labeling conditions.

SunRed™ has even better water solubility than Texas Red®, Texas Red®-X and California Red™. It is extremely useful for labeling hydrophobic oligonucleotides that are often poorly labeled by Texas Red® or Texas Red®-X. The conjugates of hydrophobic oligonucleotides with Texas Red® are difficult to use for measuring biological activity assays due to their poor water solubility.

### Features and Benefits of California Red™ and SunRed™

- *Spectral properties are almost identical to those of Texas Red®*
- *Fluorescence is less-quenched on proteins than Texas Red®*
- *More stable than Texas Red®*
- *Higher conjugation yield*

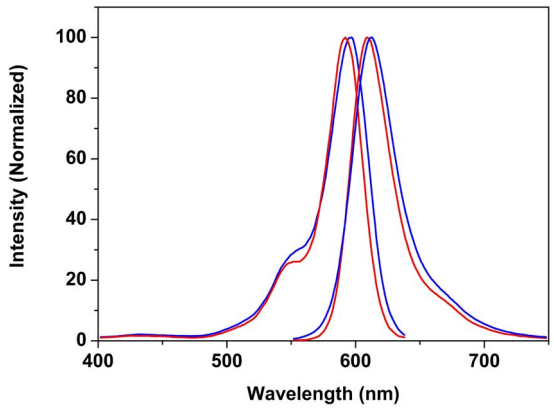


**Figure 2.8.** Spectral comparison of California Red™ and Texas Red® conjugated to $_2$HN-AAACATATAATACGG. (Red: Texas Red®; Blue: California Red™).

### Table 2.4 Comparison of California Red™ and SunRed™ with Texas Red®

| Dye Properties | California Red™ | SunRed™ | Texas Red® |
|---|---|---|---|
| Maximum Absorption Wavelength (nm) | 595 | 595 | 594* |
| Maximum Fluorescence Wavelength (nm) | 615 | 615 | 613* |
| Extinction Coefficient ($cm^{-1}M^{-1}$) | 100,000 | 100,000 | 100,000 |
| Purity | Single Isomer | Single Isomer | Mixture of 3 Isomers |
| Reactive Group | Succinimidyl Ester | Succinimidyl Ester | Sulfonyl Chloride |
| Water Solubility (pH 7.0) | <1 mg/mL | >10 mg/mL | <1 mg/mL |
| Conjugation Yield (after HPLC Purification)** | 57% | 56% | 21% |

* Glycine conjugate; ** Based on the reaction with $_2$HN-AAACATATAATACGG.

### Table 2.5 Superior Replacement Dyes for Texas Red®

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 473 | California Red™, SE | 5 mg | 583 | 603 | 100,000 | 0.456 |
| 480 | Sulforhodamine 101 Sulfonyl Chloride [also known as Texas Red®] | 10 mg | 588 | 601 | 100,000 | 0.456 |
| 472 | SunRed™, SE | 5 mg | 583 | 603 | 100,000 | 0.484 |
| 485 | Texas Red® Alkyne *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.456 |
| 484 | Texas Red® Azide *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.456 |
| 482 | Texas Red® Cadaverine *Single Isomer* | 5 mg | 582 | 602 | 95,000 | 0.456 |
| 481 | Texas Red® Hydrazide *Single Isomer* | 5 mg | 582 | 602 | 95,000 | 0.456 |
| 483 | Texas Red® Maleimide *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.456 |


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## 2.6 Cyanine Dyes

As fluorescent dyes, cyanine dyes have many uses, particularly in biomedical imaging. Depending on the structures, they cover the visible and NIR portion of the spectrum. Cy3®, Cy5® and Cy7® are the most popular cyanine dyes. Cy3® has orange fluorescence (~550/570 nm), while Cy5® is fluorescent in the red region (~650/670 nm). Cy3® and Cy5® are typically combined for 2- color detection. They are usually synthesized with reactive groups on either one or both of the nitrogen side chains so that they can be chemically linked to either nucleic acids or protein molecules. Labeling is done for visualization and quantification purposes. Cy3® and Cy5® are used in a wide variety of biological applications including comparative genomic hybridization and gene chips, which are used in transcriptomics. They are also used to label proteins and nucleic acids for various studies including proteomics and RNA localization.

Caution must be exercised when selecting a cyanine dye. In general, Cy3®, Cy5®, Cy5.5® and Cy7® are all referred as the sulfonated cyanine dyes (Figure 2.10 on page 12) in the literature. However, some vendors offer the less expensive non-sulfonated cyanines (Figure 2.9) as replacements for the Cy3®, Cy5®, Cy5.5® and Cy7® (sulfonated) cited in the literature. The widely used sulfonated cyanine dyes (known as Cy3®, Cy5®, Cy5.5® and Cy7®) should *not* be exchanged with the less expensive non-sulfonated cyanine dyes due to the drastically different properties. The sulfonated cyanine dyes have much higher fluorescence quantum yields than the non-sulfonated cyanines (Table 2.6) in aqueous solutions. The sulfonated cyanine dyes are highly water-soluble while the non-sulfonated cyanines are difficult to be dissolved in water.

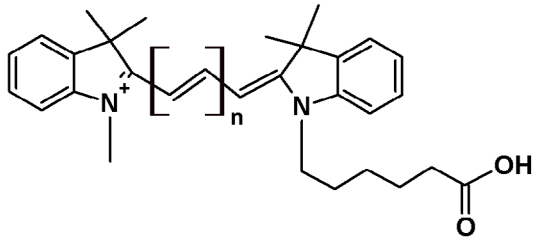


**Figure 2.9a.** The chemical structures of typical non-sulfonated cyanine dyes for labeling oligonucleotides (Cy3NS: n=1; Cy5NS: n=2; Cy7NS: n=3).

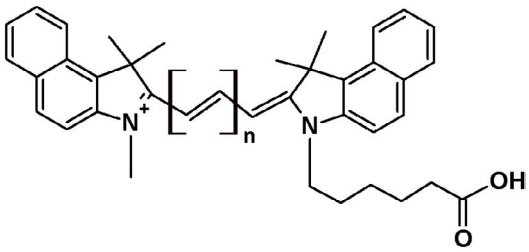


**Figure 2.9b.** The chemical structures of typical non-sulfonated cyanine dyes for labeling oligonucleotides (Cy3.5NS: n=1; Cy5.5NS: n=2; Cy7.5NS: n=3).

**Table 2.6 Spectral Comparison of Cyanine Dyes**

| Product Name | Ex (nm) | Em (nm) | EC ($cm^{-1} M^{-1}$) | Quantum Yield |
|---|---|---|---|---|
| Cy3® (Sulfonated) | 555 | 565 | 150,000 | 0.10 |
| Cy3NS (Non-Sulfonated) | 549 | 565 | 145,000 | 0.07 |
| Cy5® (Sulfonated) | 649 | 664 | 250,000 | 0.25 |
| Cy5NS (Non-Sulfonated) | 644 | 665 | 230,000 | 0.16 |
| Cy5.5® (Sulfonated) | 676 | 695 | 250,000 | 0.18 |
| Cy5.5NS (Non-Sulfonated) | 675 | 697 | 230,000 | Not Determined |
| Cy7® (Sulfonated) | 749 | 775 | 275,000 | 0.12 |
| Cy7NS (Non-Sulfonated) | 750 | 780 | 250,000 | 0.02 |

**Table 2.7 Non-Sulfonated Cyanine Dyes for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1} M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 190 | Cy3NS Acid | 100 mg | 549 | 565 | 145,000 | 0.033 |
| 191 | Cy3NS, Succinimidyl Ester | 25 mg | 549 | 565 | 145,000 | 0.033 |
| 194 | Cy5NS Acid | 100 mg | 644 | 665 | 230,000 | 0.088 |
| 195 | Cy5NS, Succinimidyl Ester | 25 mg | 644 | 665 | 230,000 | 0.088 |
| 197 | Cy7NS Acid | 100 mg | 750 | 780 | 250,000 | 0.053 |
| 198 | Cy7NS, Succinimidyl Ester | 25 mg | 750 | 780 | 250,000 | 0.053 |
| 181 | ICG-ATT [3-ICG-acyl-1,3-thiazolidine-2-thione] | 1 mg | 780 | 800 | 240,000 | 0.114 |
| 182 | ICG-OSu | 1 mg | 780 | 800 | 240,000 | 0.114 |
| 180 | ICG-Sulfo-OSu | 1 mg | 780 | 800 | 240,000 | 0.114 |

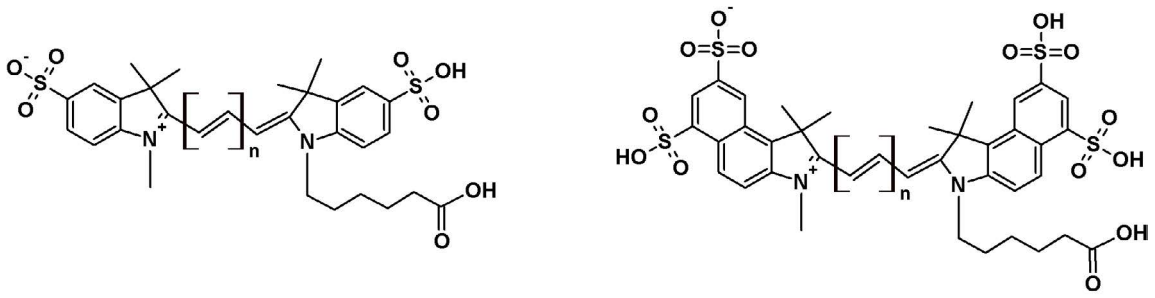

**Figure 2.10.** The chemical structures of sulfonated cyanine dyes for labeling oligonucleotides. (Left: Cy3®: n=1; Cy5®: n=2; Cy7®: n=3. Right: Cy3.5®: n=1; Cy5.5®: n= 2; Cy7.5®: n=3).



**Table 2.8 Sulfonated Cyanine Dyes for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1} M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 6070 | Cy3® Phosphoramidite | 50 µmol | 549 | 565 | 150,000 | 0.033 |
| 6071 | Cy5® Phosphoramidite | 50 µmol | 644 | 665 | 250,000 | 0.088 |
| 144 | Cyanine 3 Alkyne [equivalent to Cy3® Alkyne] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 145 | Cyanine 3 Amine [equivalent to Cy3® Amine] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 143 | Cyanine 3 Azide [equivalent to Cy3® Azide] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 146 | Cyanine 3 Hydrazide [equivalent to Cy3® Hydrazide] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 142 | Cyanine 3 Maleimide [equivalent to Cy3® Maleimide] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 141 | Cyanine 3, Monosuccinimidyl Ester [equivalent to Cy3® NHS Ester] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 139 | Cyanine 3.5 Amine [equivalent to Cy3.5® Amine] | 1 mg | 581 | 596 | 125,000 | 0.151 |
| 149 | Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide] | 1 mg | 581 | 596 | 125,000 | 0.151 |
| 148 | Cyanine 3.5, Monosuccinimidyl Ester [equivalent to Cy3.5® NHS Ester] | 1 mg | 581 | 596 | 125,000 | 0.151 |
| 154 | Cyanine 5 Alkyne [equivalent to Cy5® Alkyne] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 155 | Cyanine 5 Amine [equivalent to Cy5® Amine] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 153 | Cyanine 5 Azide [equivalent to Cy5® Azide] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 156 | Cyanine 5 Hydrazide [equivalent to Cy5® Hydrazide] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 152 | Cyanine 5 Maleimide [equivalent to Cy5® Maleimide] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 151 | Cyanine 5, Monosuccinimidyl Ester [equivalent to Cy5® NHS Ester] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 179 | Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 176 | Cyanine 5.5 Amine [equivalent to Cy5.5® Amine] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 178 | Cyanine 5.5 Azide [equivalent to Cy5.5® Azide] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 177 | Cyanine 5.5 Hydrazide [equivalent to Cy5.5® Hydrazide] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 175 | Cyanine 5.5 Maleimide [equivalent to Cy5.5® Maleimide] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 174 | Cyanine 5.5 Monosuccinimidyl Ester [equivalent to Cy5.5® NHS Ester] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 164 | Cyanine 7 Alkyne [equivalent to Cy7® Alkyne] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 165 | Cyanine 7 Amine [equivalent to Cy7® Amine] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 163 | Cyanine 7 Azide [equivalent to Cy7® Azide] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 166 | Cyanine 7 Hydrazide [equivalent to Cy7® Hydrazide] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 162 | Cyanine 7 Maleimide [equivalent to Cy7® Maleimide] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 161 | Cyanine 7, Monosuccinimidyl Ester [equivalent to Cy7® NHS Ester] | 1 mg | 749 | 776 | 275,000 | 0.025 |


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## 2.7 Tide Fluor™ Dyes Optimized for Labeling Oligos

Although EDANS, FAM, TAMRA, ROX, Cy3® and Cy5® have been widely used to develop a variety of oligonucleotide probes, there are still some limitations in the use of these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS have 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 pH dependent. They only exhibit the strongest fluorescence at higher pH. The 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.

**Table 2.9 Tide Fluor™ Dye Equivalents of Common Dyes**

| If you are using | Try this Tide Fluor™ dye |
|---|---|
| Alexa Fluor® 350, AMCA, DyLight™ 350 | TF1 [Tide Fluor™ 1] |
| Alexa Fluor® 488, Cy2®, FITC, DyLight™ 488 | TF2 [Tide Fluor™ 2] |
| Alexa Fluor® 555, Cy3®, DyLight™ 550, TRITC | TF3 [Tide Fluor™ 3] |
| | TF3WS [Tide Fluor™ 3WS] |
| Alexa Fluor® 594, DyLight™ 594, Texas Red® | TF4 [Tide Fluor™ 4] |
| Alxea Fluor® 647, Cy5®, DyLight™ 650 | TF5WS [Tide Fluor™ 5WS] |
| Alexa Fluor® 680, Cy5.5®, IRDye® 700, DyLight™ 680 | TF6WS [Tide Fluor™ 6WS] |
| Alexa Fluor® 750, Cy7®, DyLight™ 750 | TF7WS [Tide Fluor™ 7WS] |
| Alexa Fluor® 790, DyLight™ 800, IRDye® 800 | TF8WS [Tide Fluor™ 8WS] |

Among cyanine dyes, non-sulfonated Cy3® and Cy5® are occasionally used for developing a variety of oligonucleotide probes, but they have quite low fluorescence quantum yields in aqueous media. The sulfonated Cy3® and Cy5® have improved fluorescence quantum yields. Some Alexa Fluor™ dyes (e.g. Alexa Fluor® 555, 647, 680, 700 and 750) are sulfonated cyanine dyes. However, they are extremely expensive. It's unpractical to use them for preparing oligonucleotide conjugates in some cases.

To address these limitations, AAT Bioquest has developed Tide Fluor™ donor dyes that have almost identical spectral properties to those of Alexa Fluor® dyes. They are optimized as building blocks for developing FRET oligonucleotides and peptides for a variety of biological applications. We recommend you try our Tide Fluor™ dyes at much lower cost with comparable performance.

Our Tide Fluor™ dyes (such as TF1, TF2, TF3, TF4, TF5, TF6, TF7 and TF8) have stronger fluorescence and higher photostability than the typical fluorophores such as fluoresceins, rhodamines and cyanines described above. Our TF2 has the similar excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily used for the biological applications that are done with fluoresceins. 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 DyLight® dyes), Tide Fluor™ dyes are much more cost-effective with comparable or even better performance for your desired biological applications. On oligonucleotide, TF3 is much brighter and more photostable than Cy3®, Alexa Fluor® 555 and DyLight™ 555, although TF3 has almost identical spectra to those of the three dyes (Figure 2.12).

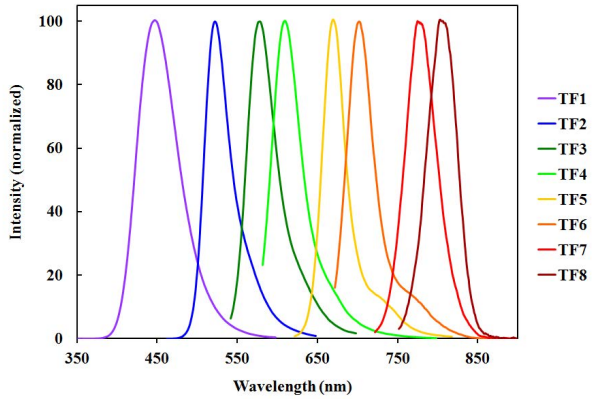


**Figure 2.11.** The normalized fluorescence spectra of Tide Fluor™ dyes.

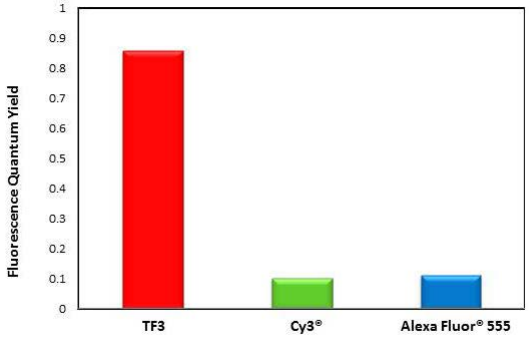


**Figure 2.12.** Fluorescence quantum yield comparison of TF3, Cy3® and Alexa Fluor® 555 .

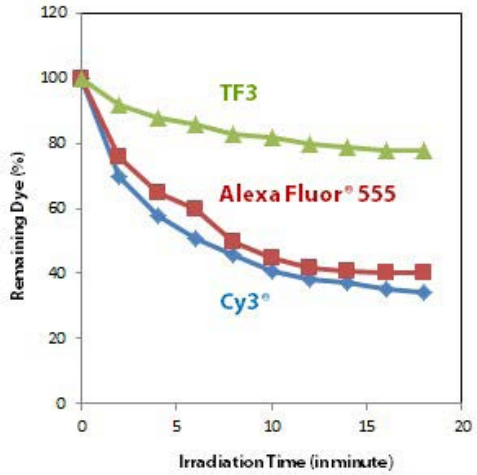


**Figure 2.13.** The photostability comparison of TF3 vs Alexa Fluor® 555 and Cy3®.

**Table 2.10 Tide Fluor™ Dyes, a Full Spectrum of Fluorescent Dyes to Replace Alexa Fluor® Dyes**

| Tide Fluor™ Donor | Ex (nm) | Em (nm) | Features and Benefits | Ordering Information |
|---|---|---|---|---|
| Tide Fluor™ 1 (TF1) | 345 | 442 | Alternative to EDANS<br>• *Much stronger absorption*<br>• *Much stronger fluorescence*<br>• *Less environmental sensitivity* | Cat# 2236 (TF1 azide, click chemistry)<br>Cat# 2237 (TF1 alkyne, click chemistry)<br>Cat# 2238 (TF1 acid)<br>Cat# 2239 (TF1 amine)<br>Cat# 2242 (TF1 maleimide, SH-reactive)<br>Cat# 2244 (TF1 SE, $NH_2$-reactive) |
| Tide Fluor™ 2 (TF2) | 500 | 527 | Alternative to FAM, FITC and Alexa Fluor® 488<br>• *pH-insensitive fluorescence*<br>• *Good photostability* | Cat# 2245 (TF2 acid)<br>Cat# 2246 (TF2 amine)<br>Cat# 2247 (TF2 maleimide, SH-reactive)<br>Cat# 2248 (TF2 SE, $NH_2$-reactive)<br>Cat# 2252 (TF2 azide, click chemistry)<br>Cat# 2253 (TF2 alkyne, click chemistry) |
| Tide Fluor™ 2WS (TF2WS) | 502 | 525 | Alternative to Alexa Fluor® 488<br>• *pH-insensitive fluorescence*<br>• *Good photostability* | Cat# 2348 (TF2WS acid)<br>Cat# 2349 (TF2WS SE, $NH_2$-reactive) |
| Tide Fluor™ 3 (TF3) | 555 | 584 | Alternative to Cy3® and Alexa Fluor® 555<br>• *Strong fluorescence*<br>• *Good photostability* | Cat# 2254 (TF3 azide, click chemistry)<br>Cat# 2255 (TF3 alkyne, click chemistry)<br>Cat# 2268 (TF3 acid)<br>Cat# 2269 (TF3 amine)<br>Cat# 2270 (TF3 maleimide, SH-reactive)<br>Cat# 2271 (TF3 SE, $NH_2$-reactive) |
| Tide Fluor™ 3WS (TF3WS) | 555 | 565 | Alternative to Cy3® and Alexa Fluor® 555<br>• *Strong fluorescence*<br>• *Good photostability* | Cat# 2345 (TF3WS acid)<br>Cat# 2346 (TF3WS SE, $NH_2$-reactive) |
| Tide Fluor™ 4 (TF4) | 590 | 618 | Alternative to ROX, Texas Red® and Alexa Fluor® 594<br>• *Strong fluorescence*<br>• *Good photostability* | Cat# 2285 (TF4 acid)<br>Cat# 2286 (TF4 amine)<br>Cat# 2287 (TF4 maleimide, SH-reactive)<br>Cat# 2289 (TF4 SE, $NH_2$-reactive)<br>Cat# 2300 (TF4 azide, click chemistry)<br>Cat# 2301 (TF4 alkyne, click chemistry) |
| Tide Fluor™ 5WS (TF5WS) | 649 | 664 | Alternative to Cy5® and Alexa Fluor® 647<br>• *Strong fluorescence*<br>• *Good photostability* | Cat# 2275 (TF5WS azide, click chemistry)<br>Cat# 2276 (TF5WS alkyne, click chemistry)<br>Cat# 2278 (TF5WS, acid)<br>Cat# 2279 (TF5WS amine)<br>Cat# 2280 (TF5WS maleimide, SH-reactive)<br>Cat# 2281 (TF5WS SE, $NH_2$-reactive) |
| Tide Fluor™ 6WS (TF6WS) | 676 | 695 | Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680<br>• *Strong fluorescence*<br>• *Photostable* | Cat# 2291 (TF6WS acid)<br>Cat# 2292 (TF6WS amine)<br>Cat# 2293 (TF6WS maleimide, SH-reactive)<br>Cat# 2294 (TF6WS SE, $NH_2$-reactive)<br>Cat# 2302 (TF6WS azide, click chemistry)<br>Cat# 2303 (TF6WS alkyne, click chemistry) |
| Tide Fluor™ 7WS (TF7WS) | 749 | 775 | Alternative to Cy7® and Alexa Fluor® 750<br>• *Strong fluorescence*<br>• *Good photostability* | Cat# 2304 (TF7WS azide, click chemistry)<br>Cat# 2305 (TF7WS alkyne, click chemistry)<br>Cat# 2330 (TF7WS acid)<br>Cat# 2331 (TF7WS amine)<br>Cat# 2332 (TF7WS maleimide, SH-reactive)<br>Cat# 2333 (TF7WS SE, $NH_2$-reactive) |
| Tide Fluor™ 8WS (TF8WS) | 775 | 807 | Alternative to IRDye® 800<br>• *Stronger fluorescence*<br>• *Higher Photostability* | Cat# 2306 (TF8WS azide, click chemistry)<br>Cat# 2307 (TF8WS alkyne, click chemistry)<br>Cat# 2335 (TF8WS acid)<br>Cat# 2336 (TF8WS amine)<br>Cat# 2337 (TF8WS maleimide, SH-reactive)<br>Cat# 2338 (TF8WS SE, $NH_2$-reactive) |


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**Table 2.11 Tide Fluor™ Dyes for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 2238 | Tide Fluor™ 1 Acid [TF1 Acid] *Superior Replacement for EDANS* | 100 mg | 345 | 442 | 20,000 | 0.246 |
| 2237 | Tide Fluor™ 1 Alkyne [TF1 Alkyne] *Superior Replacement for EDANS* | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2239 | Tide Fluor™ 1 Amine [TF1 Amine] *Superior Replacement for EDANS* | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2236 | Tide Fluor™ 1 Azide [TF1 Azide] *Superior Replacement for EDANS* | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2348 | Tide Fluor™ 2WS Acid [TF2WS Acid] *Superior Replacement for FITC* | 10 mg | 502 | 525 | 75,000 | 0.211 |
| 2349 | Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC* | 5 mg | 502 | 525 | 75,000 | 0.211 |
| 2345 | Tide Fluor™ 3WS Acid [TF3WS Acid] *Superior Replacement for Cy3®* | 10 mg | 555 | 565 | 150,000 | 0.079 |
| 2346 | Tide Fluor™ 3WS, Succinimidyl Ester [TF3WS, SE] *Superior Replacement for Cy3®* | 5 mg | 555 | 565 | 150,000 | 0.079 |
| 2285 | Tide Fluor™ 4 Acid [TF4 Acid] *Superior Replacement for ROX and Texas Red®* | 10 mg | 590 | 618 | 90,000 | 0.489 |
| 2301 | Tide Fluor™ 4 Alkyne [TF4 Alkyne] *Superior Replacement for ROX and Texas Red®* | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2286 | Tide Fluor™ 4 Amine [TF4 Amine] *Superior Replacement for ROX and Texas Red®* | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2300 | Tide Fluor™ 4 Azide [TF4 Azide] *Superior Replacement for ROX and Texas Red®* | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2287 | Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®* | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2289 | Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®* | 5 mg | 590 | 618 | 90,000 | 0.489 |
| 2278 | Tide Fluor™ 5WS Acid [TF5WS Acid] *Superior Replacement for Cy5®* | 10 mg | 649 | 664 | 250,000 | 0.023 |
| 2276 | Tide Fluor™ 5WS Alkyne [TF5WS Alkyne] *Superior Replacement for Cy5®* | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2279 | Tide Fluor™ 5WS Amine [TF5WS Amine] *Superior Replacement for Cy5®* | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2275 | Tide Fluor™ 5WS Azide [TF5WS Azide] *Superior Replacement for Cy5®* | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2280 | Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®* | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2281 | Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®* | 5 mg | 649 | 664 | 250,000 | 0.023 |
| 2291 | Tide Fluor™ 6WS Acid [TF6WS Acid] *Superior Replacement for Cy5.5®* | 10 mg | 676 | 695 | 220,000 | 0.111 |
| 2303 | Tide Fluor™ 6WS Alkyne [TF6WS Alkyne] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2292 | Tide Fluor™ 6WS Amine [TF6WS Amine] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2302 | Tide Fluor™ 6WS Azide [TF6WS Azide] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2293 | Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2294 | Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2330 | Tide Fluor™ 7WS Acid [TF7WS Acid] *Superior Replacement for Cy7®* | 10 mg | 749 | 775 | 275,000 | 0.009 |
| 2305 | Tide Fluor™ 7WS Alkyne [TF7WS Alkyne] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2331 | Tide Fluor™ 7WS Amine [TF7WS Amine] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2304 | Tide Fluor™ 7WS Azide [TF7WS Azide] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2332 | Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2333 | Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2335 | Tide Fluor™ 8WS Acid [TF8WS Acid] *Near Infrared Emission* | 10 mg | 775 | 807 | 250,000 | 0.103 |
| 2307 | Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2336 | Tide Fluor™ 8WS Amine [TF8WS Amine] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2306 | Tide Fluor™ 8WS Azide [TF8WS Azide] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2337 | Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2338 | Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |

**Table 2.12 Dye Selection Guide for Preparing Fluorescent Oligonucleotides with Desired Excitation and Emission Properties***

| Emisson Color / Excitation Wavelength | Blue | Green | Yellow | Orange | Red | Far Red | Infra-Red |
|---|---|---|---|---|---|---|---|
| 355 nm | Alexa Fluor® 350<br>AMCA<br>**EDANS**<br>iFluor™ 350<br>**Tide Fluor™ 1** | Dansyl Dyes | | | | | |
| 405 nm | Alexa Fluor® 405<br>**iFluor™ 405**<br>mFlour™ Violet 450 | mFlour™ Violet 510 | mFlour™ Violet 540 | | | | |
| 436 nm | Alexa Fluor® 430<br>iFluor™ 405<br>**mFlour™ Violet 450** | mFlour™ Violet 510 | mFlour™ Violet 540 | | | | |
| 488 nm | | Alexa Fluor® 488<br>Cy2®<br>**FAM**<br>FITC<br>iFluor™ 488<br>**Tide Fluor™ 2**<br>**Tide Fluor™ 2WS** | | | mFlour™ Blue 570 | | |
| 532 nm | | | | Alexa Fluor® 532<br>iFluor™ 532<br>**Rhodamine 6G** | mFlour™ Green 620 | | |
| 561 nm | | | | | Alexa Fluor® 555<br>**California Red™**<br>Cy3®<br>iFluor™ 555<br>mFlour™ Yellow 630<br>ROX<br>**TAMRA**<br>Texas Red®<br>Texas Red®-X<br>**Tide Fluor™ 3**<br>**Tide Fluor™ 4** | | |
| 633 nm | | | | | | Alexa Fluor® 647<br>Cy5®<br>iFluor™ 647<br>**Tide Fluor™ 5** | mFlour™ Red 780 |
| 647 nm | | | | | | Alexa Fluor® 647<br>Cy5®<br>iFluor™ 647<br>**Tide Fluor™ 5** | mFlour™ Red 780 |
| 670 nm | | | | | | Alexa Fluor® 680<br>Cy5.5®<br>iFluor™ 680<br>**Tide Fluor™ 6** | mFlour™ Red 780 |
| 745 nm | | | | | | | Alexa Fluor® 750<br>Cy7®<br>iFluor™ 750<br>IRDye® 800<br>**Tide Fluor™ 7** |

* Notes: 1). Excitation sources: 355 nm: UV laser/mercury arc lamp; 405 nm: violet diode laser; 436 nm: mercury arc lamp; 488 nm: argon laser; 532 nm: Nd: YAG laser; 561 nm: yellow diode laser; 633 nm: He-Ne laser; 647 nm: krypton laser; 670 nm: NIR laser; 745 nm: NIR laser.
2). Recommended dyes are bolded in green based on the cost and performance.


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# Non-Fluorescent Quenchers for Labeling Oligonucleotides

3

## 3.1 FRET Oligonucleotides and Their Applications

The ability of fluorescent compounds to transfer energy absorbed from light to nearby molecules, a process called fluorescence resonance energy transfer (FRET), has been exploited for the development of homogeneous methods of nucleic acid detection. These FRET techniques are being used to monitor the progress of nucleic acid amplification reactions in real time. They are also useful for imaging gene expression in living cells.

FRET oligo techniques are used in a few different ways. (1). A pair of interactive fluorophores is attached to the ends of two different oligo probes or to the two ends of the same oligo probe. A target nucleic acid reveals itself by either bringing the donor fluorophore and the acceptor fluorophore close to each other, permitting FRET between them to occur, or by separating them from each other, precluding FRET. (2). With two complimentary oligo probes, one of them serves as a probe for a single-stranded target sequence. The 5' end of one oligo is labeled with a donor fluorophore and the 3' end of the other oligo is labeled with an acceptor fluorophore, such that when the two oligos are annealed to each other, the two labels are close to one another. Since small complementary oligos bind to each other in a dynamic equilibrium, target strands compete for binding to the probe, causing the separation of the labeled oligos. (3). The donor and acceptor fluorophores are attached to the two ends of the same oligo, which serves as the probe. Since an oligo in solution behaves like a random coil, its ends occasionally come close to one another, resulting in a measurable change in FRET. However, when the probe binds to its target, the rigidity of the probe–target helix keeps the two ends of the probe apart from each other, precluding interaction between the donor and the acceptor moieties. (4). Single-stranded oligo is served as a molecular beacon that possesses short additional sequences at either end of a probe sequence that are complementary to each another, enabling terminal labels to be in close proximity through the formation of a hairpin stem. Binding of this probe to its target creates a relatively rigid probe–target hybrid that causes the disruption of the hairpin stem and the removal of the donor moiety from the vicinity of the acceptor moiety, thus restoring the fluorescence of the donor. (5). In addition to these hybridization-based schemes and their variations, dual-labeled randomly coiled probes that bind to template strands during PCR, can be enzymatically cleaved by the 5'→3' endonuclease activity of DNA polymerase (TaqMan probes), separating the donor and acceptor moieties and enabling nucleic acid synthesis to be monitored in real time.

Oligos labeled with a pair of interactive fluorophores have proven to be useful in real-time monitoring of a number of other reactions involving nucleic acids. If an acceptor fluorophore is brought closer to a donor fluorophore within the range 20–100 Å, the intensity of the fluorescence of the acceptor fluorophore increases, whereas the intensity of the fluorescence of the donor fluorophore decreases. This is due to the increase in the efficiency of FRET from the donor to the acceptor fluorophore. However, if the two moieties are brought any closer, the fluorescence intensities of both the donor fluorophore and the acceptor fluorophore are reduced. At the intimate distances, most of the absorbed energy is dissipated as heat and only a small amount of energy is emitted as light, a phenomenon sometimes referred to as static or contact quenching.

A further simplification of homogeneous assays that utilize fluorescently labeled probes is the use of non-fluorescent dyes as acceptors or quenchers, such as our Tide Quencher™ dyes. Quenching by non-fluorescent dyes enables changes in fluorescence intensity to be measured directly, rather than as an alteration in the shape of the emission spectrum, which is more difficult to monitor. This improvement has also led to a higher degree of multiplexing, as the part of the spectrum that would have been occupied by the fluorescence of the quencher can instead be reserved for the fluorescence of additional fluorophores for the detection of more targets.

## 3.2 Non-Fluorescent DABCYL Dyes

DABCYL dyes are quite useful for preparing FRET oligonucleotides. AAT Bioquest offers a variety of DABCYL derivatives for developing FRET oligonucleotide substrates. However, DABCYL is not an effective quencher for longer wavelength fluorescent dyes (such as rhodamines and cyanines). We recommend you try our Tide Quencher™ dyes optimized for preparing FRET oligonucleotides to get superior performance.

**Table 3.1 DABCYL Derivatives for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ab (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|
| 2001 | DABCYL Acid | 5 g | 426 | 20,000 | 0.614 |
| 2006 | DABCYL C2 Amine | 100 mg | 428 | 20,000 | 0.614 |
| 2008 | DABCYL C2 Maleimide | 25 mg | 428 | 20,000 | 0.614 |
| 2004 | DABCYL, Succinimidyl Ester | 1 g | 453 | 20,000 | 0.614 |
| 2005 | DABCYL, Succinimidyl Ester | 5 g | 453 | 20,000 | 0.614 |

## 3.3 Tide Quencher™ Dyes Optimized to Maximize FRET Efficiency

Although DABCYL has been used to develop a variety of FRET applications, its low quenching efficiency for 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 robust Tide Quencher™ acceptor dyes for the development of longer wavelength FRET probes. Tide Quencher™ dyes are a great choice to eliminate the limitations of classic quenchers.


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Tide Quencher™ series of nonfluorescent dyes cover the full visible spectrum with unusually high efficiency. For example, TQ2 has absorption maximum perfectly matching the emission of FAM while TQ3, TQ5 and TQ7 are proven to be the best quenchers for Cy3®, Cy5® and Cy7®. Tide Quencher™ dyes are excellent dark quenchers that are individually optimized to pair with all the popular fluorescent dyes such as fluoresceins, rhodamines and cyanines. These Tide Quencher™ dark FRET acceptors (such as TQ1, TQ2, TQ3, TQ4, TQ5, TQ6 and TQ7) are perfect 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.

### The Advantages of Tide Quencher™ Dyes:

- *TQ dyes enable you to explore the FRET potentials that might be impossible with other quenchers.*
- *Versatile reactive forms are convenient for self-constructing your desired FRET biomolecules.*
- *Perfectly match your desired fluorescent donors.*
- *Competitive price with better performance.*

### Table 3.2 Tide Quencher™ Dye Equivalents of Common Dyes

| If you are using | Try this Tide Quencher™ dye |
|---|---|
| DABCYL, BHQ®-0, QSY®35 | TQ1 [Tide Quencher™ 1] |
| BHQ®-1, QXL™ 520, QSY®35 | TQ2 [Tide Quencher™ 2] |
| BHQ®-2 | TQ3 [Tide Quencher™ 3] |
| BHQ®-3, QSY®7, QSY®9 | TQ4 [Tide Quencher™ 4] |
| QSY®21 | TQ5 [Tide Quencher™ 5] |
| IRDye® QC-1 | TQ6 [Tide Quencher™ 6] |
| IRDye® QC-1 | TQ7 [Tide Quencher™ 7] |

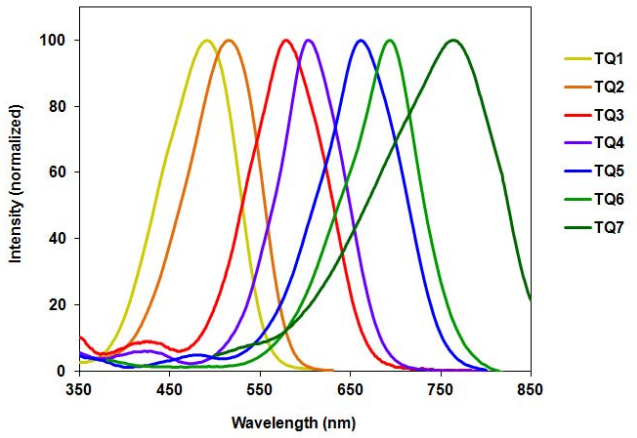


**Figure 3.1.** The normalized absorption spectra of TQ1, 2, 3, 4, 5, 6 and 7.

### Table 3.3. Recommended FRET Pairs for Developing FRET Oligonucleotides to Detect Nucleic Acids*

| Donors \ Acceptors | DABCYL | TQ1 | TQ2 | TQ3 | TQ4 | TQ5 | TQ6 | TQ7 |
|---|---|---|---|---|---|---|---|---|
| EDANS | +++++ | +++++ | +++ | | | | | |
| MCA | +++++ | +++++ | +++ | | | | | |
| TF1 | +++++ | +++++ | +++ | | | | | |
| FAM/FITC | +++ | +++ | +++++ | +++ | | | | |
| Cy2®/TF2 | +++ | +++ | +++++ | +++ | | | | |
| HEX/JOE/TET | | | +++ | +++++ | +++ | | | |
| Cy3®/TAMRA/TF3 | | | +++ | +++++ | +++ | | | |
| ROX/Texas Red® | | | | +++ | +++++ | +++ | | |
| TF4 | | | | +++ | +++++ | +++ | | |
| Cy5®/TF5 | | | | | +++ | +++++ | +++ | |
| Cy5.5®/TF6 | | | | | | +++ | +++++ | +++ |
| Cy7®/TF7 | | | | | | | +++ | +++++ |

* +++++ Best to use; +++ OK to use; Not recommended to use.

**Table 3.4 Tide Quencher™ Dyes for Developing FRET-Based Real Time PCR Probes**

| Dark FRET Acceptor | $\lambda_{max}$ (nm) | Features and Benefits | Ordering Information |
|---|---|---|---|
| Tide Quencher™ 1 (TQ1) | 490 | Alternative to Dabcyl, QSY® 35 and BHQ®-0<br>• *Best paired with Tide Fluor™ 1 (TF1)*<br>• *Excellent FRET efficiency with coumarins* | Cat# 2188 (TQ1 azide, click chemistry)<br>Cat# 2189 (TQ1 alkyne, click chemistry)<br>Cat# 2190 (TQ1 acid)<br>Cat# 2192 (TQ1 amine)<br>Cat# 2193 & 2194 (TQ1 CPG, OH-reactive)<br>Cat# 2196 (TQ1 maleimide, SH-reactive)<br>Cat# 2198 (TQ1 phosphoramidite, OH-reactive)<br>Cat# 2199 (TQ1 SE, $NH_2$-reactive) |
| Tide Quencher™ 2 (TQ2) | 515 | Alternative to BHQ®-1<br>• *Best paired with Tide Fluor™ 2 (TF2)*<br>• *Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers* | Cat# 2211 (TQ2 azide, click chemistry)<br>Cat# 2212 (TQ2 alkyne, click chemistry)<br>Cat# 2200 (TQ2 acid)<br>Cat# 2202 (TQ2 amine)<br>Cat# 2203 & 2204 (TQ2 CPG, OH-reactive)<br>Cat# 2206 (TQ2 maleimide, SH-reactive)<br>Cat# 2208 (TQ2 phosphoramidite, OH-reactive)<br>Cat# 2210 (TQ2 SE, $NH_2$-reactive) |
| Tide Quencher™ 2WS (TQ2WS) | 515 | Alternative to BHQ®-1<br>• *Best paired with Tide Fluor™ 2 (TF2)*<br>• *Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers* | Cat# 2050 (TQ2WS acid)<br>Cat# 2058 (TQ2WS SE, $NH_2$-reactive) |
| Tide Quencher™ 3 (TQ3) | 570 | Alternative to QSY® 7, QSY® 9 and BHQ®-2<br>• *Best paired with Tide Fluor™ 3 (TF3)*<br>• *Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers* | Cat# 2220 (TQ3 acid)<br>Cat# 2222 (TQ3 amine)<br>Cat# 2223 & 2224 (TQ3 CPG, OH-reactive)<br>Cat# 2226 (TQ3 maleimide, SH-reactive)<br>Cat# 2228 (TQ3 phosphoramidite, OH-reactive)<br>Cat# 2230 (TQ3 SE, $NH_2$-reactive)<br>Cat# 2231 (TQ3 azide, click chemistry)<br>Cat# 2232 (TQ3 alkyne, click chemistry) |
| Tide Quencher™ 3WS (TQ3WS) | 578 | Alternative to QSY® 7, QSY® 9 and BHQ®-2<br>• *Best paired with Tide Fluor™ 3 (TF3)*<br>• *Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers* | Cat# 2227 (TQ3WS acid)<br>Cat# 2229 (TQ3WS SE, $NH_2$-reactive) |
| Tide Quencher™ 4 (TQ4) | 603 | • *Strong absorption*<br>• *Best paired with Tide Fluor™ 4 (TF4)*<br>• *Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers* | Cat# 2062 & 2063 (TQ4 CPG, OH-reactive) |
| Tide Quencher™ 4WS (TQ4WS) | ~590 | • *Strong absorption*<br>• *Best paired with Tide Fluor™ 4 (TF4)*<br>• *Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers* | Cat# 2060 (TQ4WS acid)<br>Cat# 2061 (TQ4WS amine)<br>Cat# 2064 (TQ4WS maleimide, SH-reactive)<br>Cat# 2067 (TQ4WS SE, $NH_2$-reactive)<br>Cat# 2068 (TQ4WS azide, click chemistry)<br>Cat# 2069 (TQ4WS alkyne, click chemistry) |
| Tide Quencher™ 5 (TQ5) | ~670 | Alternative to QSY® 21 and BHQ®-3<br>• *Best paired with Tide Fluor™ 5 (TF5)*<br>• *Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa luor® 647* | Cat# 2077 & 2078 (TQ5 CPG, OH-reactive) |
| Tide Quencher™ 5WS (TQ5WS) | ~670 | Alternative to QSY® 21 and BHQ®-3<br>• *Best paired with Tide Fluor™ 5 (TF5)*<br>• *Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa Fluor® 647* | Cat# 2075 (TQ5WS acid)<br>Cat# 2076 (TQ5WS amine)<br>Cat# 2079 (TQ5WS maleimide, SH-reactive)<br>Cat# 2081 (TQ5WS SE, $NH_2$-reactive)<br>Cat# 2082 (TQ5WS azide, click chemistry)<br>Cat # 2083 (TQ5WS alkyne, click chemistry) |
| Tide Quencher™ 6WS (TQ6WS) | ~700 | • *Stronger absorption*<br>• *Best paired with Tide Fluor™ 6 (TF6)*<br>• *Better FRET efficiency with Cy5.5®, IRDye® 700 and Alexa Fluor® 680 than other commercial quenchers* | Cat# 2090 (TQ6WS acid)<br>Cat# 2091 (TQ6WS amine)<br>Cat# 2094 (TQ6WS maleimide, SH-reactive)<br>Cat# 2096 (TQ6WS SE, $NH_2$-reactive)<br>Cat# 2097 (TQ6WS azide, click chemistry)<br>Cat# 2098 (TQ6WS alkyne, click chemistry) |
| Tide Quencher™ 7WS (TQ7WS) | ~760 | • *Stronger absorption*<br>• *Best paired with Tide Fluor™ 7 (TF7)*<br>• *Better FRET efficiency with Cy7® and Alexa Fluor® 750 than other commercial quenchers* | Cat# 2105 (TQ7WS acid)<br>Cat# 2106 (TQ7WS amine)<br>Cat# 2109 (TQ7WS maleimide, SH-reactive)<br>Cat# 2111 (TQ7WS SE, $NH_2$-reactive)<br>Cat# 2112 (TQ7WS azide, click chemistry)<br>Cat# 2113 (TQ7WS alkyne, click chemistry) |


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**Table 3.5 Tide Quencher™ Dyes for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ab | EC ($cm^{-1} M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|
| 2189 | Tide Quencher™ 1 Alkyne [TQ1 Alkyne] | 5 mg | 490 | 20,000 | 0.147 |
| 2188 | Tide Quencher™ 1 Azide [TQ1 Azide] | 5 mg | 490 | 20,000 | 0.147 |
| 2193 | Tide Quencher™ 1 CPG [TQ1 CPG] *500 Å* | 100 mg | 490 | 20,000 | 0.147 |
| 2194 | Tide Quencher™ 1 CPG [TQ1 CPG] *1000 Å* | 100 mg | 490 | 20,000 | 0.147 |
| 2196 | Tide Quencher™ 1 Maleimide [TQ1 Maleimide] | 5 mg | 490 | 20,000 | 0.147 |
| 2199 | Tide Quencher™ 1, Succinimidyl Ester [TQ1, SE] | 25 mg | 490 | 20,000 | 0.147 |
| 2212 | Tide Quencher™ 2 Alkyne [TQ2 Alkyne] | 100 mg | 515 | 21,000 | 0.100 |
| 2211 | Tide Quencher™ 2 Azide [TQ2 Azide] | 5 mg | 515 | 21,000 | 0.100 |
| 2203 | Tide Quencher™ 2 CPG [TQ2 CPG] *500 Å* | 100 mg | 515 | 21,000 | 0.100 |
| 2204 | Tide Quencher™ 2 CPG [TQ2 CPG] *1000 Å* | 100 mg | 515 | 21,000 | 0.100 |
| 2206 | Tide Quencher™ 2 Maleimide [TQ2 Maleimide] | 5 mg | 515 | 21,000 | 0.100 |
| 2210 | Tide Quencher™ 2, Succinimidyl Ester [TQ2, SE] | 25 mg | 515 | 21,000 | 0.100 |
| 2058 | Tide Quencher™ 2WS, Succinimidyl Ester [TQ2WS, SE] | 5 mg | 515 | 19,000 | 1.296 |
| 2232 | Tide Quencher™ 3 Alkyne [TQ3 Alkyne] | 5 mg | 570 | 22,000 | 0.085 |
| 2231 | Tide Quencher™ 3 Azide [TQ3 Azide] | 5 mg | 570 | 22,000 | 0.085 |
| 2223 | Tide Quencher™ 3 CPG [TQ3 CPG] *500 Å* | 100 mg | 570 | 22,000 | 0.085 |
| 2224 | Tide Quencher™ 3 CPG [TQ3 CPG] *1000 Å* | 100 mg | 570 | 22,000 | 0.085 |
| 2226 | Tide Quencher™ 3 Maleimide [TQ3 Maleimide] | 5 mg | 570 | 22,000 | 0.085 |
| 2230 | Tide Quencher™ 3, Succinimidyl Ester [TQ3, SE] | 25 mg | 570 | 22,000 | 0.085 |
| 2229 | Tide Quencher™ 3WS, Succinimidyl Ester [TQ3WS, SE] | 1 mg | 578 | 90,000 | 0.186 |
| 2062 | Tide Quencher™ 4 CPG [TQ4 CPG] *500 Å* | 100 mg | 603 | 22,000 | 0.146 |
| 2063 | Tide Quencher™ 4 CPG [TQ4 CPG] *1000 Å* | 100 mg | 603 | 22,000 | 0.146 |
| 2069 | Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne] | 1 mg | 603 | 90,000 | 0.149 |
| 2068 | Tide Quencher™ 4WS Azide [TQ4WS Azide] | 1 mg | 603 | 90,000 | 0.149 |
| 2064 | Tide Quencher™ 4WS Maleimide [TQ4WS Maleimide] | 1 mg | 603 | 90,000 | 0.149 |
| 2067 | Tide Quencher™ 4WS, Succinimidyl Ester [TQ4WS, SE] | 1 mg | 603 | 90,000 | 0.149 |
| 2077 | Tide Quencher™ 5 CPG [TQ5 CPG] *500 Å* | 100 mg | 661 | 22,000 | 0.170 |
| 2078 | Tide Quencher™ 5 CPG [TQ5 CPG] *1000 Å* | 100 mg | 661 | 22,000 | 0.170 |
| 2083 | Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne] | 1 mg | 661 | 130,000 | 0.072 |
| 2076 | Tide Quencher™ 5WS Amine [TQ5WS Amine] | 1 mg | 661 | 130,000 | 0.072 |
| 2082 | Tide Quencher™ 5WS Azide [TQ5WS Azide] | 1 mg | 661 | 130,000 | 0.072 |
| 2079 | Tide Quencher™ 5WS Maleimide [TQ5WS Maleimide] | 1 mg | 661 | 130,000 | 0.072 |
| 2081 | Tide Quencher™ 5WS, Succinimidyl Ester [TQ5WS, SE] | 1 mg | 661 | 130,000 | 0.072 |
| 2098 | Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne] | 1 mg | 704 | 130,000 | 0.120 |
| 2097 | Tide Quencher™ 6WS Azide [TQ6WS Azide] | 1 mg | 704 | 130,000 | 0.120 |
| 2094 | Tide Quencher™ 6WS Maleimide [TQ6WS Maleimide] | 1 mg | 704 | 130,000 | 0.120 |
| 2096 | Tide Quencher™ 6WS, Succinimidyl Ester [TQ6WS, SE] | 1 mg | 704 | 130,000 | 0.120 |
| 2113 | Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne] | 1 mg | 763 | 140,000 | 0.072 |
| 2112 | Tide Quencher™ 7WS Azide [TQ7WS Azide] | 1 mg | 763 | 140,000 | 0.072 |
| 2109 | Tide Quencher™ 7WS Maleimide [TQ7WS Maleimide] | 1 mg | 763 | 140,000 | 0.072 |
| 2111 | Tide Quencher™ 7WS, Succinimidyl Ester [TQ7WS, SE] | 1 mg | 763 | 140,000 | 0.072 |

# 4 Dyes for Labeling Amino-Modified Oligonucleotides




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Amine-reactive fluorescent probes are widely used to modify amino-modified oligonucleotides at the added amino residue. A number of fluorescent amino-reactive dyes have been developed to label various oligonucleotides, and the resultant conjugates are widely used in biological applications. Two major classes of amine-reactive fluorescent reagents are currently used to label oligonucleotides: succinimidyl esters (SE) and sulfonyl chlorides(SC).

AAT Bioquest offers all the popular amine-reactive fluorescent dyes for labeling oligonucleotides. In general, the chemical reactions of succinimidyl esters give higher yields. We strongly recommend that you choose succinimidyl esters if other conditions and factors are equal.

## 4.1 Dye Succinimidyl Esters

Succinimidyl esters (SE) are proven to be the best reagents for amine modifications because the amide bonds formed are generally stable. There are a few factors that need 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 amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, thus amine acylation reactions are usually carried out above pH 7.5. Oligonucleotide modifications by succinimidyl esters can typically be done at pH 7.5-8.5.

***Reaction Buffers:*** Buffers that contain free amines, such as Tris, glycine and thiol compounds, must be avoided when using an amine-reactive reagent.

***Reaction Temperature:*** Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.


**Figure 4.1.** The reaction scheme of an amino-modified oligonucleotide with a dye succinimidyl ester.

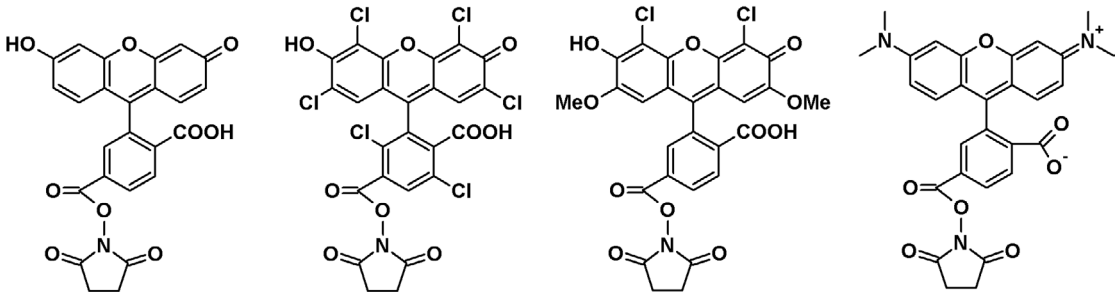


**Figure 4.2.** The chemical structures of some typical dye succinimidyl esters.

**Table 4.1 Dye Succinimidyl Esters for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 502 | AMCA, Succinimidyl Ester | 10 mg | 353 | 455 | 19,000 | 0.183 |
| 473 | California Red™ SE | 5 mg | 583 | 603 | 75,000 | 0.456 |
| 141 | Cyanine 3, Monosuccinimidyl Ester [equivalent to Cy3® NHS Ester] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 148 | Cyanine 3.5, Monosuccinimidyl Ester [equivalent to Cy3.5® NHS Ester] | 1 mg | 581 | 596 | 125,000 | 0.151 |
| 151 | Cyanine 5, Monosuccinimidyl Ester [equivalent to Cy5® NHS Ester] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 174 | Cyanine 5.5, Monosuccinimidyl Ester [equivalent to Cy5.5® NHS Ester] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 161 | Cyanine 7, Monosuccinimidyl Ester [equivalent to Cy7® NHS Ester] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 506 | DEAC, SE [7-Diethylaminocoumarin-3-carboxylic Acid, Succinimidyl Ester] | 25 mg | 432 | 472 | 40,000 | Not Determined |
| 110 | 5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Mixed Isomers* | 25 mg | 494 | 519 | 75,000 | 0.255 |
| 116 | 6-FAM, SE [6-Carboxyfluorescein, Succinimidyl Ester] *Single Isomer* | 10 mg | 495 | 517 | 75,000 | 0.255 |
| 202 | 6-HEX, SE [6-Carboxy-2',4,4',5',7,7'-hexachlorofluorescein, Succinimidyl Ester] *Single Isomer* | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 556 | 7-Hydroxy-4-methylcoumarin-3-acetic Acid, Succinimidyl Ester | 25 mg | 364 | 458 | 25,000 | 0.168 |
| 551 | 7-Hydroxycoumarin-3-carboxylic Acid, Succinimidyl Ester | 50 mg | 363 | 447 | 35,000 | 0.226 |
| 553 | 7-Hydroxycoumarin-4-acetic Acid, Succinimidyl Ester | 25 mg | 360 | 450 | 18,000 | Not Determined |
| 203 | 6-JOE, SE [6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, Succinimidyl Ester] | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 558 | MCA Succinimidyl Ester [7-Methoxycoumarin-4-acetic Acid, Succinimidyl Ester] | 25 mg | 322 | 390 | 15,000 | 0.134 |
| 563 | 7-Methoxycoumarin-3-carboxylic Acid, Succinimidyl Ester | 100 mg | 358 | 410 | 25,000 | 0.071 |
| 390 | 5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, Succinimidyl Ester] *Mixed Isomers* | 25 mg | 576 | 601 | 93,000 | 0.301 |
| 392 | 6-ROX, SE | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 478 | SunRed™ SE | 1 mg | 583 | 603 | 100,000 | 0.484 |
| 370 | 5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] | 25 mg | 546 | 575 | 78,000 | 0.320 |
| 376 | 6-TAMRA, SE [6-Carboxytetramethylrhodamine, Succinimidyl Ester] *Single Isomer* | 5 mg | 547 | 573 | 78,000 | 0.335 |
| 211 | 6-TET, SE [6-Carboxy-2',4,7',7-tetrachlorofluorescein, Succinimidyl Ester] | 5 mg | 521 | 536 | 78,000 | 0.191 |
| 2244 | Tide Fluor™ 1, Succinimidyl Ester [TF1, SE]*Superior Replacement for EDANS* | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2248 | Tide Fluor™ 2, Succinimidyl Ester [TF2, SE]*Superior Replacement for Fluorescein* | 5 mg | 500 | 527 | 75,000 | 0.288 |
| 2349 | Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC* | 5 mg | 502 | 525 | 75,000 | 0.211 |
| 2271 | Tide Fluor™ 3, Succinimidyl Ester [TF3, SE] *Superior Replacement for Cy3®* | 5 mg | 555 | 584 | 75,000 | 0.331 |
| 2346 | Tide Fluor™ 3WS, Succinimidyl Ester [TF3WS, SE] *Superior Replacement for Cy3®* | 5 mg | 555 | 565 | 150,000 | 0.079 |
| 2289 | Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®* | 5 mg | 590 | 618 | 90,000 | 0.489 |
| 2281 | Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®* | 5 mg | 649 | 664 | 250,000 | 0.023 |
| 2294 | Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2333 | Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 250,000 | 0.009 |
| 2338 | Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |


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## 4.2 Dye Sulfonyl Chlorides

Sulfonyl chlorides (SC) are highly reactive. These reagents are unstable in water, especially at the higher pH required for reactions with aliphatic amines. Molecular modifications by sulfonyl chlorides need to be carefully carried out preferably at low temperature. Sulfonyl chlorides can also react with phenols (such as tyrosine), aliphatic alcohols (such as polysaccharide), thiols (such as cysteine) and imidazoles (such as histidine), but these reactions are not common in proteins or in aqueous solutions. There are a few factors that need be considered when SC compounds are used for conjugation reactions:

- ***Solvents:*** SC dyes are generally hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF). They are unstable in dimethylsulfoxide (DMSO) and should never be used in this solvent.

- ***Reaction pH:*** The labeling reactions of amines with SC reagents are strongly pH dependent. SC reagents react with non-protonated amine groups. On the other hand, the sulfonylation reagents tend to hydrolyze in the presence of water, with the hydrolyzing rate increasing as the pH increases. Thus sulfonylation-based conjugations may require a pH value ranging from 9.0 to 10.0 for optimal conjugations. In general, sulfonylation-based conjugations have much lower yields than the succinimidyl ester-based conjugations.

- ***Reaction Buffers:*** Buffers that contain free amines such as Tris and glycine must be avoided when using an amine-reactive reagent. High concentrations of nucleophilic thiol compounds should also be avoided because they may react with the labeling reagent to form unstable intermediates that could destroy the reactive dye.

- ***Reaction Temperature:*** Most SC conjugations are done at room temperature. However, reduced temperature may be required for a particular SC labeling reaction.

Among dye sulfonyl chlorides, Texas Red® is the most common one. However, there are a few limitations with Texas Red®:

- ***Difficult Purification:*** As a mixture of three isomers, the purification of Texas Red® is quite hard;

- ***Slow Reaction:*** The progression of the reaction is quite slow just like other SC dyes in general.

We have developed two excellent Texas Red® replacements: California Red™ and SunRed™. They have essentially identical spectra and give much higher conjugation yields. Compared to Texas Red®, California Red™ has much higher labeling efficiency, and more importantly the resulted conjugate is more fluorescent than the corresponding Texas Red® conjugate. Our in-house studies indicated that California Red™ is more stable than Texas Red® under the same labeling conditions.

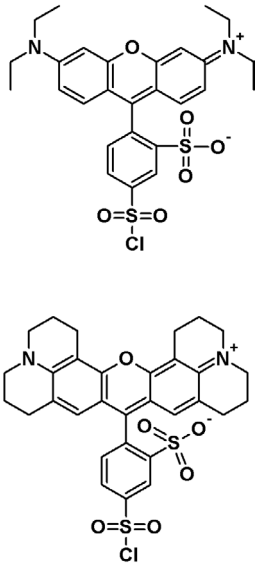

**Figure 4.3.** The chemical structures of Lissamine rhodamine B sulfonyl chloride (Cat# 470, top) and Texas Red® (Cat# 480, bottom).

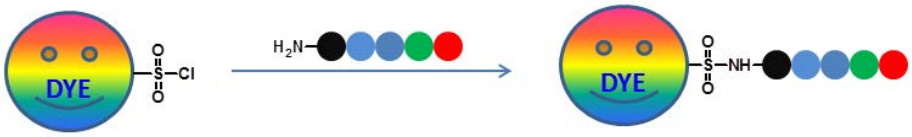


**Figure 4.4.** The reaction scheme of a amino-modified oligonucleotide with a dye sulfonyl chloride.

### Table 4.2 Dye Sulfonyl Chlorides for Labeling Oligonucleotides

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 473 | California Red™ SE | 5 mg | 583 | 603 | 100,000 | 0.456 |
| 470 | Lissamine Rhodamine B Sulfonyl Chloride | 100 mg | 568 | 583 | 95,000 | 0.288 |
| 471 | LRB Red™ SE | 10 mg | 568 | 583 | 95,000 | 0.288 |
| 480 | Sulforhodamine 101 Sulfonyl Chloride [Texas Red®] | 10 mg | 588 | 601 | 95,000 | 0.456 |

# 5 Dyes for Labeling Thiol-Modified Oligonucleotides




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Because free thiol (SH) groups, also called mercapto groups, are not present as abundantly as other groups in most oligonucleotides, thiol-reactive reagents often provide a means of selectively modifying an oligonucleotide at a defined site. Therefore thiol-reactive dyes are often used to prepare fluorescent oligonucleotides for probing biological structures, functions and interactions. There are many types of thiol-reactive dyes reported in the literature, including iodoacetamides, disulfides, maleimides, vinyl sulfones and various electron-deficient aryl halides and sulfonates. Maleimides are by far the most popular thiol-reactive moiety. They readily react with thiol moieties of biopolymers to form very stable thioether conjugates even under neutral conditions. Maleimides require conjugation conditions less stringent than those of iodoacetamides, and do not react with histidines and methionines under physiological conditions. For example, most conjugations can be done at room temperature and neutral pH.

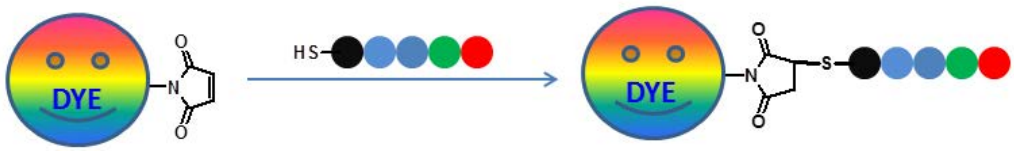


**Figure 5.1.** The reaction scheme of a thiol-modified oligonucleotide with a dye maleimide.

## Table 5.1 Dye Maleimides for Labeling Oligonucleotides

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 503 | AMCA C2 Maleimide | 5 mg | 353 | 455 | 18,000 | 0.183 |
| 142 | Cyanine 3 Maleimide [equivalent to Cy3® Maleimide] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 149 | Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide] | 1 mg | 581 | 596 | 125,000 | 0.151 |
| 152 | Cyanine 5 Maleimide [equivalent to Cy5® Maleimide] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 175 | Cyanine 5.5 Maleimide [equivalent to Cy5.5® Maleimide] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 162 | Cyanine 7 Maleimide [equivalent to Cy7® Maleimide] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 2008 | DABCYL C2 Maleimide | 25 mg | 428 | N/A | 20,000 | 0.614 |
| 2026 | DNP Maleimide | 25 mg | 350 | N/A | 18,000 | Not Determined |
| 617 | EDANS C2 Maleimide | 25 mg | 335 | 493 | 5,500 | 2.44 |
| 2242 | Tide Fluor™ 1 Maleimide [TF1 Maleimide] *Superior Replacement for EDANS* | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2247 | Tide Fluor™ 2 Maleimide [TF2 Maleimide] *Superior Replacement for fluorescein* | 1 mg | 500 | 527 | 75,000 | 0.288 |
| 2270 | Tide Fluor™ 3 Maleimide [TF3 Maleimide] *Superior Replacement for Cy3®* | 1 mg | 555 | 584 | 75,000 | 0.331 |
| 2287 | Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®* | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2280 | Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®* | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2293 | Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®* | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2332 | Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®* | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2337 | Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission* | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2196 | Tide Quencher™ 1 Maleimide [TQ1 Maleimide] | 5 mg | 490 | N/A | 20,000 | 0.147 |
| 2206 | Tide Quencher™ 2 Maleimide [TQ2 Maleimide] | 5 mg | 515 | N/A | 21,000 | 0.100 |
| 2226 | Tide Quencher™ 3 Maleimide [TQ3 Maleimide] | 5 mg | 570 | N/A | 22,000 | 0.085 |
| 2064 | Tide Quencher™ 4WS Maleimide [TQ4WS Maleimide] | 1 mg | 603 | N/A | 90,000 | 0.149 |
| 2079 | Tide Quencher™ 5WS Maleimide [TQ5WS Maleimide] | 1 mg | 661 | N/A | 130,000 | 0.072 |
| 2094 | Tide Quencher™ 6WS Maleimide [TQ6WS Maleimide] | 1 mg | 704 | N/A | 130,000 | 0.120 |
| 2109 | Tide Quencher™ 7WS Maleimide [TQ7WS Maleimide] | 1 mg | 763 | N/A | 140,000 | 0.072 |
| 423 | 5(6)-TAMRA C6 Maleimide | 5 mg | 544 | 575 | 75,000 | 0.320 |
| 425 | 6-TAMRA C6 Maleimide | 5 mg | 544 | 575 | 75,000 | 0.335 |

# 6 Clickable Dyes for Labeling Oligonucleotides




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"Click Chemistry" is a term introduced by K. B. Sharpless in 2001 to describe reactions that are high in yields, wide in scope, and create only by-products that can be removed without chromatography. Click chemistry reactions are stereospecific, simple to perform and can be conducted in easily removable or benign solvents. This concept was developed in parallel with the interest within the pharmaceutical, material, and other industries in capabilities of generating large libraries of compounds for screening in discovery research. Several types of reaction have been identified that fulfill these criteria. They are thermodynamically-favored reactions that lead specifically to one product, such as nucleophilic ring opening reactions of epoxides and aziridines; non-aldol type carbonyl reactions, such as formation of hydrazones and heterocycles; eletrophilic additions to carbon-carbon multiple bonds, such as oxidative formation of epoxides and Michael Additions; and cycloaddition reactions.

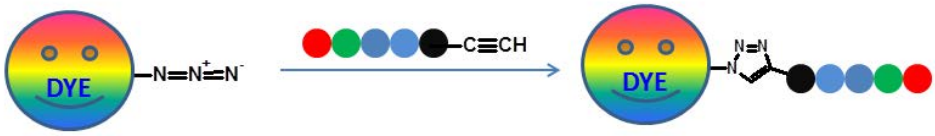


**Figure 6.1.** The reaction scheme of an alkyne-modified oligonucleotide with a dye azide.



**Table 6.1 Dye Azides for Labeling Oligonucleotides**

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 508 | AMCA Azide | 1 mg | 353 | 455 | 18,000 | 0.183 |
| 143 | Cyanine 3 Azide [equivalent to Cy3® Azide] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 153 | Cyanine 5 Azide [equivalent to Cy5® Azide] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 178 | Cyanine 5.5 Azide [equivalent to Cy5.5® Azide] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 163 | Cyanine 7 Azide [equivalent to Cy7® Azide] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 133 | 6-FAM Azide | 10 mg | 494 | 521 | 75,000 | 0.255 |
| 240 | 6-HEX Azide | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 1091 | iFluor™ 647 Azide | 1 mg | 649 | 665 | 250,000 | 0.025 |
| 248 | 6-JOE Azide | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 494 | 6-ROX Azide | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 490 | 6-TAMRA Azide | 5 mg | 547 | 573 | 75,000 | 0.335 |
| 244 | 6-TET Azide | 5 mg | 521 | 536 | 76,000 | 0.191 |
| 484 | Texas Red® Azide *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.436 |
| 2236 | Tide Fluor™ 1 Azide [TF1 Azide] | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2252 | Tide Fluor™ 2 Azide [TF2 Azide] | 1 mg | 500 | 527 | 75,000 | 0.288 |
| 2254 | Tide Fluor™ 3 Azide [TF3 Azide] | 1 mg | 555 | 584 | 75,000 | 0.331 |
| 2300 | Tide Fluor™ 4 Azide [TF4 Azide] | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2275 | Tide Fluor™ 5WS Azide [TF5WS Azide] | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2302 | Tide Fluor™ 6WS Azide [TF6WS Azide] | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2304 | Tide Fluor™ 7WS Azide [TF7WS Azide] | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2306 | Tide Fluor™ 8WS Azide [TF8WS Azide] | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2188 | Tide Quencher™ 1 Azide [TQ1 Azide] | 5 mg | 515 | N/A | 20,000 | 0.147 |
| 2211 | Tide Quencher™ 2 Azide [TQ2 Azide] | 5 mg | 515 | N/A | 21,000 | 0.100 |
| 2231 | Tide Quencher™ 3 Azide [TQ3 Azide] | 5 mg | 570 | N/A | 22,000 | 0.085 |
| 2068 | Tide Quencher™ 4WS Azide [TQ4WS Azide] | 1 mg | 603 | N/A | 90,000 | 0.149 |
| 2082 | Tide Quencher™ 5WS Azide [TQ5WS Azide] | 1 mg | 661 | N/A | 130,000 | 0.072 |
| 2097 | Tide Quencher™ 6WS Azide [TQ6WS Azide] | 1 mg | 704 | N/A | 130,000 | 0.120 |
| 2112 | Tide Quencher™ 7WS Azide [TQ7WS Azide] | 1 mg | 763 | N/A | 140,000 | 0.072 |

An examination of the azide-alkyne cycloaddition shows that it fulfills many of the prerequisites. The copper-catalyzed azide–alkyne cyloaddition is a two-step process. First, one reaction partner—either an azide or alkyne linked to a "building block" such as an oligonucleotide, is incorporated by conventional synthesis. Subsequently, the other reaction partner—the complementary alkyne or azide linked to a fluorescent dye, biotin or other detection reagent—is "clicked" into place in the presence of catalytic copper (I). One reaction partner must be an azide derivative and the other one must be an alkyne derivative, but either functional moiety can serve as the incorporated molecule or the detection molecule. The reaction is also regiospecific, yielding exclusively 1,4-disubstituted-1,2,3-triazole linkages. The 1,2,3-triazole linkage between an oligonucleotide and a dye is extremely stable. It is not susceptible to hydrolysis, oxidation or reduction. AAT Bioquest offers a variety of dye azides and alkynes for labeling oligonucleotides.

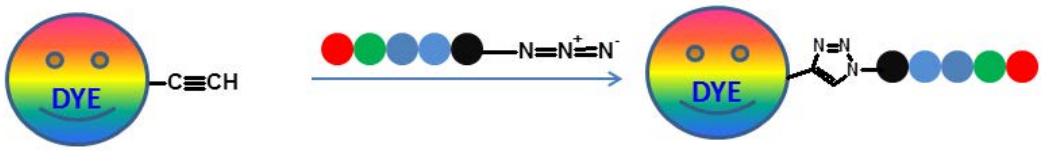


**Figure 6.2.** The reaction scheme of azide-modified oligonucleotide with a dye alkyne.



### Table 6.2 Dye Alkynes for Labeling Oligonucleotides

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 507 | AMCA Alkyne | 1 mg | 353 | 455 | 18,000 | 0.183 |
| 144 | Cyanine 3 Alkyne [equivalent to Cy3® Alkyne] | 1 mg | 555 | 565 | 150,000 | 0.042 |
| 154 | Cyanine 5 Alkyne [equivalent to Cy5® Alkyne] | 1 mg | 649 | 665 | 250,000 | 0.026 |
| 179 | Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne] | 1 mg | 678 | 701 | 230,000 | 0.094 |
| 164 | Cyanine 7 Alkyne [equivalent to Cy7® Alkyne] | 1 mg | 749 | 776 | 275,000 | 0.025 |
| 134 | 6-FAM Alkyne | 10 mg | 494 | 521 | 75,000 | 0.255 |
| 241 | 6-HEX Alkyne | 5 mg | 533 | 550 | 74,000 | 0.300 |
| 249 | 6-JOE Alkyne | 5 mg | 520 | 548 | 73,000 | 0.326 |
| 495 | 6-ROX Alkyne | 5 mg | 575 | 602 | 95,000 | 0.307 |
| 491 | 6-TAMRA Alkyne | 5 mg | 547 | 573 | 75,000 | 0.335 |
| 245 | 6-TET Alkyne | 5 mg | 521 | 536 | 76,000 | 0.191 |
| 485 | Texas Red® Alkyne *Single Isomer* | 5 mg | 588 | 601 | 95,000 | 0.436 |
| 2237 | Tide Fluor™ 1 Alkyne [TF1 Alkyne] | 5 mg | 345 | 442 | 20,000 | 0.246 |
| 2253 | Tide Fluor™ 2 Alkyne [TF2 Alkyne] | 1 mg | 500 | 527 | 75,000 | 0.288 |
| 2255 | Tide Fluor™ 3 Alkyne [TF3 Alkyne] | 1 mg | 555 | 584 | 75,000 | 0.331 |
| 2301 | Tide Fluor™ 4 Alkyne [TF4 Alkyne] | 1 mg | 590 | 618 | 90,000 | 0.489 |
| 2276 | Tide Fluor™ 5WS Alkyne [TF5WS Alkyne] | 1 mg | 649 | 664 | 250,000 | 0.023 |
| 2303 | Tide Fluor™ 6WS Alkyne [TF6WS Alkyne] | 1 mg | 676 | 695 | 220,000 | 0.111 |
| 2305 | Tide Fluor™ 7WS Alkyne [TF7WS Alkyne] | 1 mg | 749 | 775 | 275,000 | 0.009 |
| 2307 | Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] | 1 mg | 775 | 807 | 250,000 | 0.103 |
| 2189 | Tide Quencher™ 1 Alkyne [TQ1 Alkyne] | 5 mg | 515 | N/A | 20,000 | 0.147 |
| 2212 | Tide Quencher™ 2 Alkyne [TQ2 Alkyne] | 5 mg | 515 | N/A | 21,000 | 0.100 |
| 2232 | Tide Quencher™ 3 Alkyne [TQ3 Alkyne] | 5 mg | 570 | N/A | 22,000 | 0.085 |
| 2069 | Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne] | 1 mg | 603 | N/A | 90,000 | 0.149 |
| 2083 | Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne] | 1 mg | 661 | N/A | 130,000 | 0.072 |
| 2098 | Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne] | 1 mg | 704 | N/A | 130,000 | 0.120 |
| 2113 | Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne] | 1 mg | 763 | N/A | 140,000 | 0.072 |


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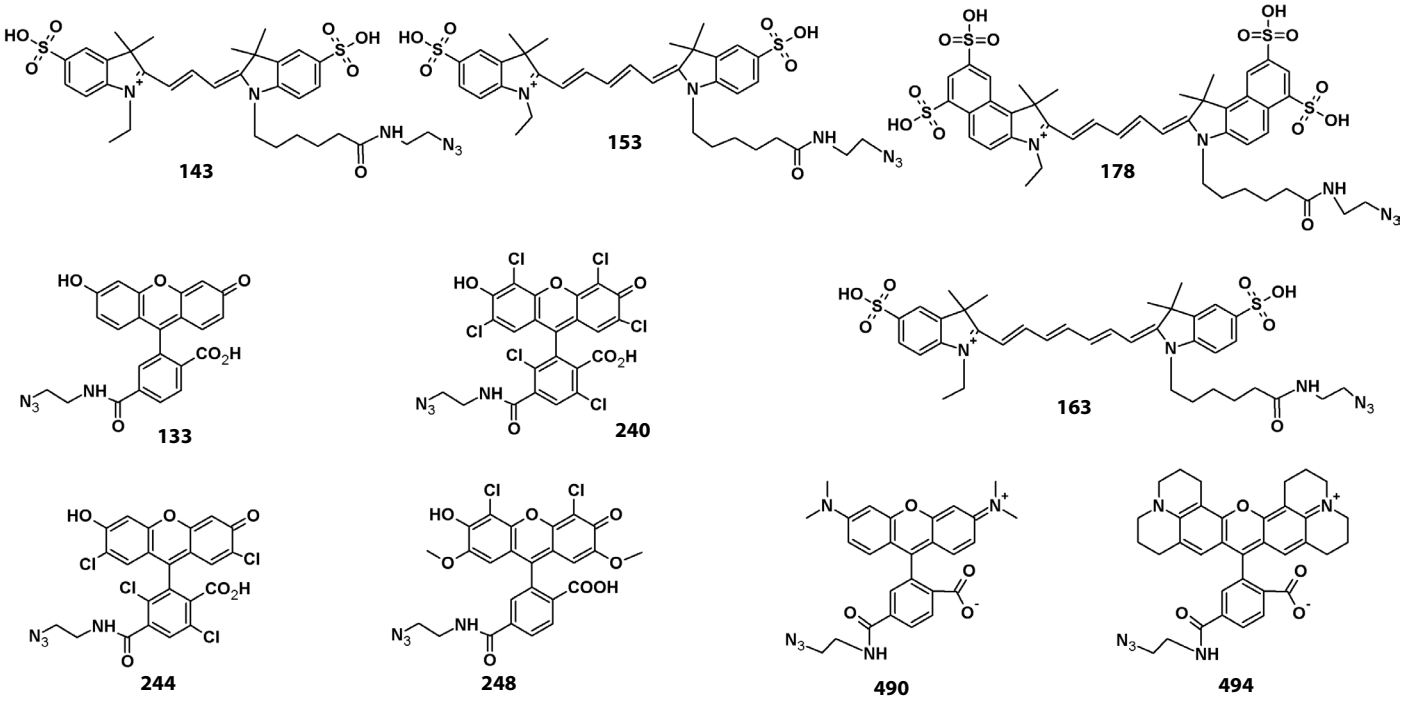


**Figure 6.3.** The chemical structures of dye azides.

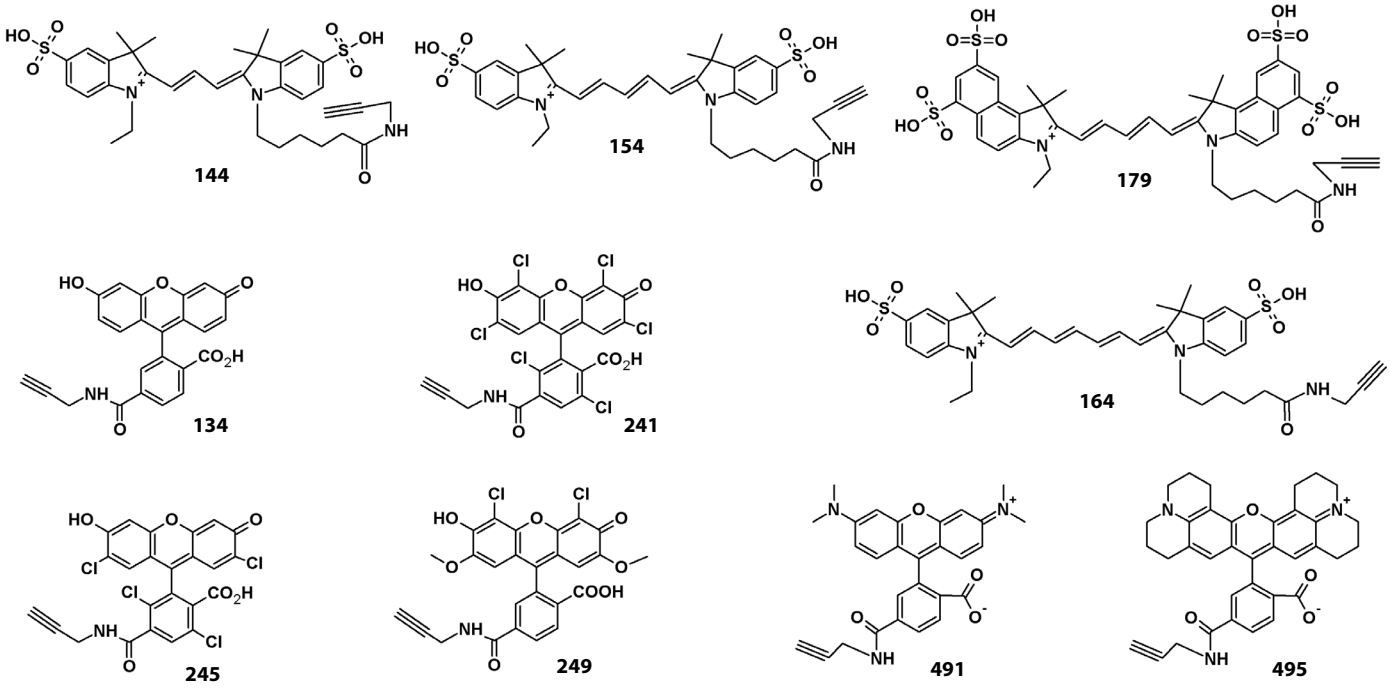


**Figure 6.4.** The chemical structures of dye alkynes.

# 7 Dye CPGs for Modifying Oligonucleotides




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AAT Bioquest's Tide Quencher™ CPGs are designed for use in DNA synthesizers to functionalize the 3'-terminus of the target oligonucleotide. Our Tide Quencher™ CPGs cover the full UV-Vis spectrum and can be paired with all the existing fluorescent dyes, such as coumarins, cyanines, fluoresceins, rhodamines as well as our outstanding Tide Fluor™ dyes (TF dyes). Tide Quencher™ dyes are excellent replacements for Black Hole Quencher® dyes (BHQ® dyes). Our Tide Quencher™ 1 (TQ1), Tide Quencher™ 2 (TQ2) and Tide Quencher™ 3 (TQ3) have equivalent performance to BHQ®-0, 1 and 2 respectively, and in some cases they have superior performance to BHQ® dyes. Our Tide Quencher™ 4 (TQ4) and Tide Quencher™ 5 (TQ5) have significantly higher quenching efficiency to longer wavelength fluorescent dyes (such as Cy5®, Alexa Fluor® 647, DyLight™ 647, iFluor™ 647 and Tide Fluor™ 5 (TF5) than BHQ®-3 due to the better overlap of TQ dye absorption spectra with the emission spectra of Cy5®, Alexa Fluor® 647, DyLight™ 647, iFluor™ 647 and TF5. TQ dye-based FRET oligonucleotides have been used as fluorescent probes for genetic research and molecular diagnostic tools for detecting infectious diseases and monitoring food safety.

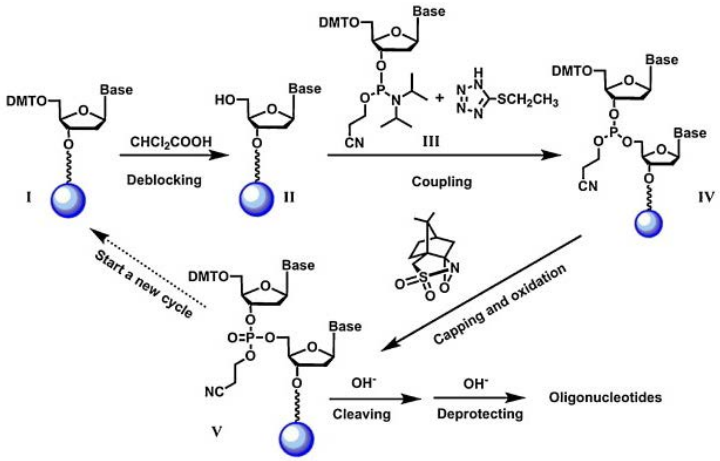


**Figure 7.1.** Solid phase oligonucleotide synthesis

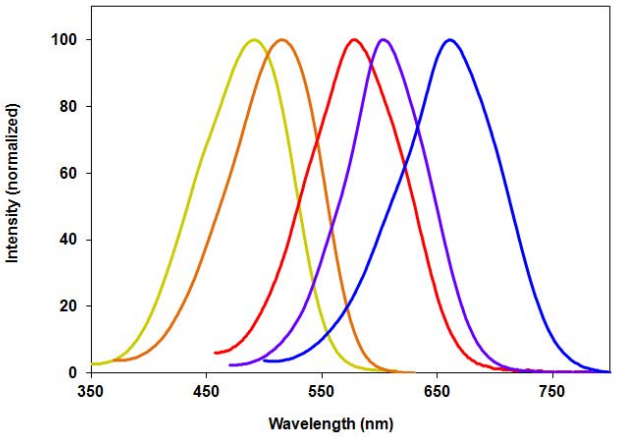


**Figure 7.2.** Normalized absorption spectra of TQ1 (yellow), TQ2 (orange), TQ3 (Red), TQ4 (purple) and TQ5 (blue).

## Table 7.1 Dye CPGs for Solid Phase Oligonucleotide Synthesis

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 6008 | 3'-DABCYL CPG *1000 Å* | 1 g | 428 | N/A | 20,000 | 0.614 |
| 6011 | 3'-Fluorescein CPG *1000 Å* | 1 g | 494 | 522 | 75,000 | 0.255 |
| 6014 | 3'-(6-Fluorescein) CPG *1000 Å* | 1 g | 494 | 522 | 75,000 | 0.255 |
| 6051 | 6-TAMRA CPG *1000 Å* | 1 g | 546 | 578 | 75,000 | 0.335 |
| 2193 | Tide Quencher™ 1 CPG [TQ1 CPG] *500 Å* | 100 mg | 490 | N/A | 20,000 | 0.147 |
| 2194 | Tide Quencher™ 1 CPG [TQ1 CPG] *1000 Å* | 100 mg | 490 | N/A | 20,000 | 0.147 |
| 2203 | Tide Quencher™ 2 CPG [TQ2 CPG] *500 Å* | 100 mg | 515 | N/A | 21,000 | 0.100 |
| 2204 | Tide Quencher™ 2 CPG [TQ2 CPG] *1000 Å* | 100 mg | 515 | N/A | 21,000 | 0.100 |
| 2223 | Tide Quencher™ 3 CPG [TQ3 CPG] *500 Å* | 100 mg | 570 | N/A | 22,000 | 0.085 |
| 2224 | Tide Quencher™ 3 CPG [TQ3 CPG] *1000 Å* | 100 mg | 570 | N/A | 22,000 | 0.085 |
| 2062 | Tide Quencher™ 4 CPG [TQ4 CPG] *500 Å* | 100 mg | 603 | N/A | 22,000 | 0.146 |
| 2063 | Tide Quencher™ 4 CPG [TQ4 CPG] *1000 Å* | 100 mg | 603 | N/A | 22,000 | 0.146 |
| 2077 | Tide Quencher™ 5 CPG [TQ5 CPG] *500 Å* | 100 mg | 661 | N/A | 22,000 | 0.170 |
| 2078 | Tide Quencher™ 5 CPG [TQ5 CPG] *1000 Å* | 100 mg | 661 | N/A | 22,000 | 0.170 |

# 8 Dye Phosphoramidites for Modifying Oligonucleotides




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AAT Bioquest's dye phosphoramidites are designed for use in DNA synthesizers to functionalize the 5'-terminus of the target oligonucleotide. They are available with a variety of chain lengths to fit exactly the desired applications. Our dye phosphoramidite selection covers the full UV-Vis spectrum with both fluorescent dyes and non-fluorescent quenchers, including coumarins, cyanines, fluoresceins, rhodamines as well as our outstanding Helix Fluor™, Tide Fluor™ and Tide Quencher™ dyes. Tide Fluor™ dyes cover the full UV-Vis spectrum as a full set of fluorescent probes, and are optimized for labeling oligonucleotides and peptides as the excellent replacements for cost-prohibitive Alexa Fluor® and DyLight™ dyes. Our Tide Fluor™ dyes have equivalent or superior performance to Alexa Fluor® or DyLight™ dyes for most biological detections.

Tide Quencher™ 1 (TQ1), Tide Quencher™ 2 (TQ2) and Tide Quencher™ 3 (TQ3) have equivalent performance to BHQ®-0, 1 and 2 respectively, and in some cases they have superior performance to BHQ® dyes. Our Tide Quencher™ 4 (TQ4) and Tide Quencher™ 5 (TQ5) have significantly higher quenching efficiency to longer wavelength fluorescent dyes (such as Cy5®, Alexa Fluor® 647, DyLight™ 647, iFluor™ 647 and Tide Fluor™ 5) than BHQ®-3 due to the better overlap of TQ dye absorption spectra with the emission spectra of Cy5®, Alexa Fluor® 647, DyLight™ 647, iFluor™ 647 and Tide Fluor™ 5. TQ dye-based FRET oligonucleotides have been used as fluorescent probes for genetic research and as molecular diagnostic tools for detecting infection diseases and monitoring food safety.

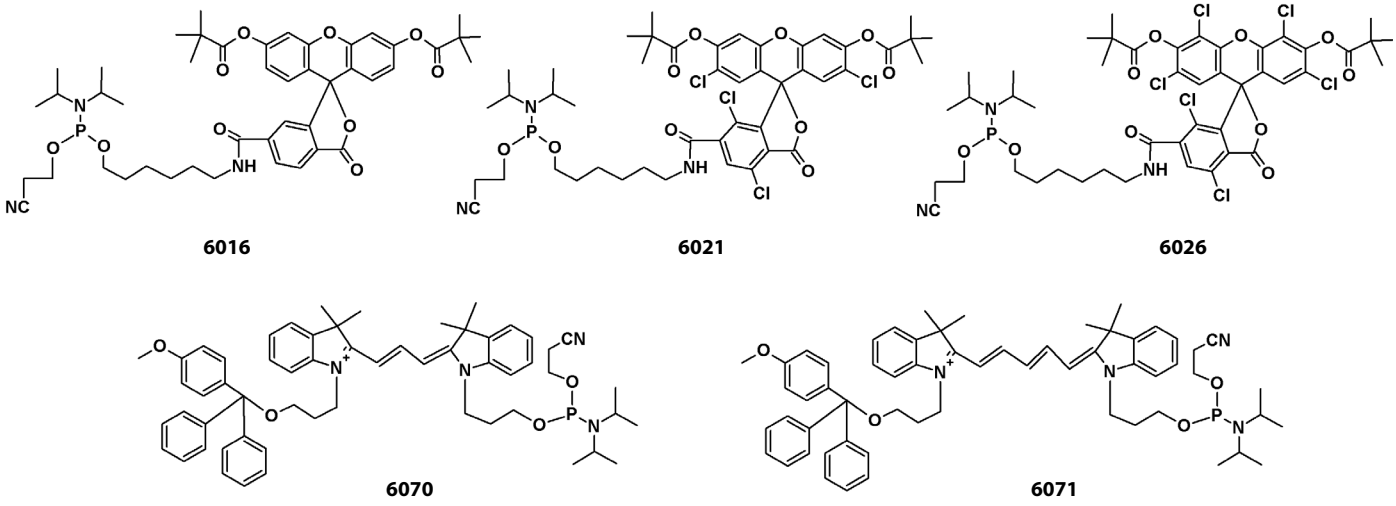


**Figure 8.1** The chemical structures of some dye phosphoramidites.

## Table 8.1 Dye Phosphoramidites for Solid Phase Oligonucleotide Synthesis

| Cat. # | Product Name | Size | Ex (nm) | Em (nm) | EC ($cm^{-1}M^{-1}$) | CF @260 nm |
|---|---|---|---|---|---|---|
| 6070 | Cy3® Phosphoramidite | 50 µmoles | 549 | 565 | 150,000 | 0.033 |
| 6071 | Cy5® Phosphoramidite | 50 µmoles | 644 | 665 | 250,000 | 0.088 |
| 6009 | 5'-DABCYL C6 Phosphoramidite | 1 g | 428 | N/A | 20,000 | 0.614 |
| 6016 | 6-FAM Phosphoramidite [5'-Fluorescein phosphoramidite] | 100 µmoles | 494 | 522 | 75,000 | 0.255 |
| 6018 | 6-Fluorescein Phosphoramidite | 100 µmoles | 494 | 522 | 75,000 | 0.255 |
| 6045 | Helix Fluor™ 6-JOE Phosphoramidite | 50 µmoles | 520 | 548 | 73,000 | 0.326 |
| 6026 | 6-HEX Phosphoramidite [5'-Hexachlorofluorescein phosphoramidite] | 100 µmoles | 535 | 553 | 74,000 | 0.300 |
| 6021 | 6-TET Phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite] | 50 µmoles | 521 | 541 | 76,000 | 0.191 |
| 6027 | 6-TET Phosphoramidite [5'-Tetrachlorofluorescein phosphoramidite] | 100 µmoles | 521 | 541 | 76,000 | 0.191 |
| 2198 | Tide Quencher™ 1 Phosphoramidite [TQ1 phosphoramidite] | 100 µmoles | 490 | N/A | 20,000 | 0.147 |
| 2208 | Tide Quencher™ 2 Phosphoramidite [TQ2 phosphoramidite] | 100 µmoles | 515 | N/A | 21,000 | 0.100 |
| 2228 | Tide Quencher™ 3 Phosphoramidite [TQ3 phosphoramidite] | 100 µmoles | 570 | N/A | 22,000 | 0.085 |

# 9 Index




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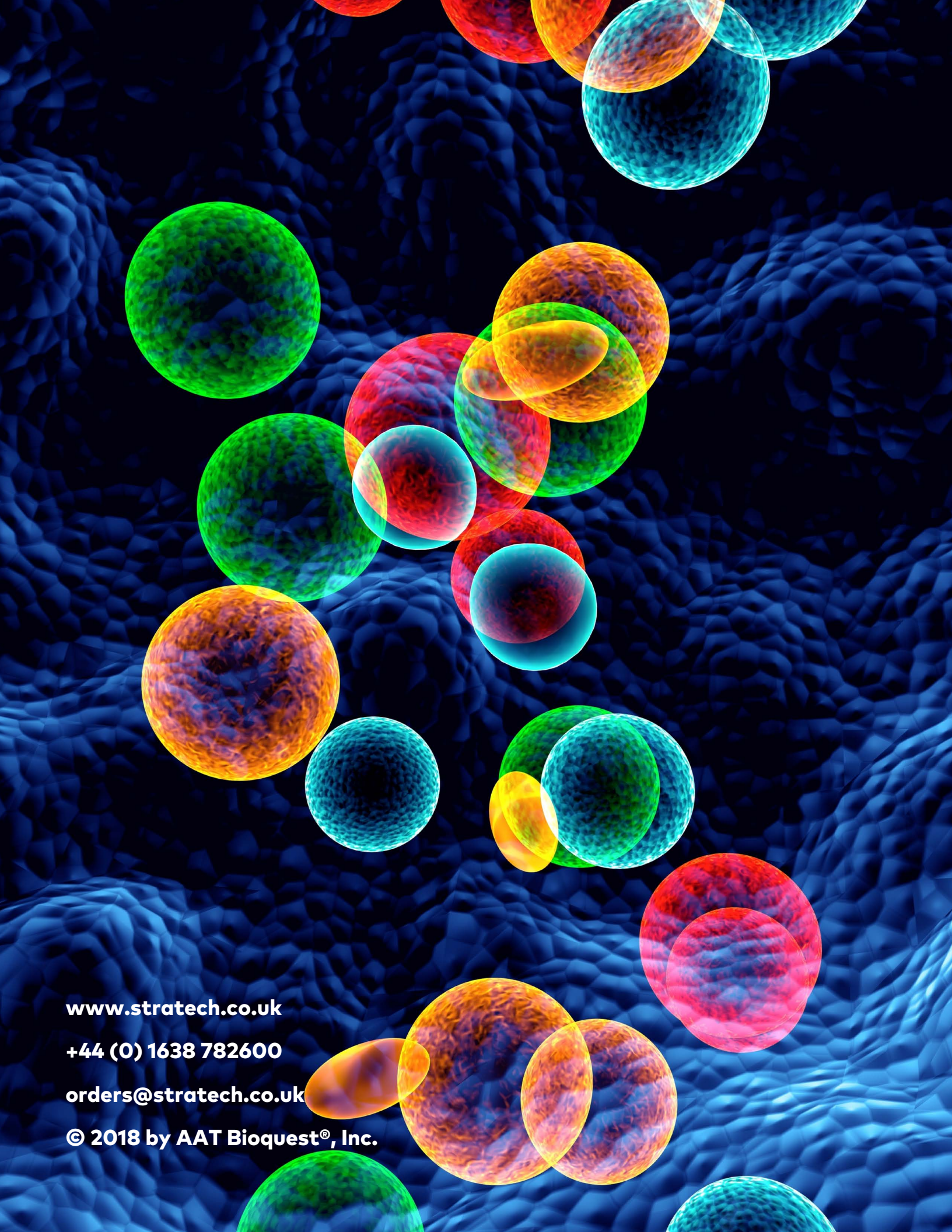

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