Peptide Labeling Reagents

Dyes | FRET Building Blocks | Quenchers

Advion Interchim scientific

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

Amino Acid Symbols:

Ala (A): Alanine
Arg (R): Arginine
Asn (N): Asparagine
Asp (D): Aspartic acid
Cys (C): Cysteine
Glu (E): Glutamic acid
Gln (Q): Glutamine
Gly (G): Glycine
His (H): Histidine
Ile (I): Isoleucine
Leu (L): Leucine
Lys (K): Lysine
Met (M): Methionine
Phe (F): Phenylalanine
Pro (P): Proline
Ser (S): Serine
Thr (T): Threonine
Trp (W): Tryptophan
Tyr (Y): Tyrosine
Val (V): Valine

Other Abbreviations Used in This Catalog

Ab: Absorption
CF: Correction factor
DMF: Dimethylformamide
DMSO: Dimethylsulfoxide
EC: Extinction coefficient
Em: Emission
Ex: Excitation
FAM: Carboxyfluorescein
FITC: Fluorescein isothiocyanate
FRET: Fluorescence resonance energy transfer
SE: Succinimidyl ester
TF: Tide Fluor™
TRF: Time resolved fluorescence
TQ: Tide Quencher™

Trademarks of AAT Bioquest

AAT Bioquest®
Bodi Fluor™
California Red™
iFluor™
LRB Red™
mFluor™
SunRed™
Tide Fluor™
Tide Quencher™

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Bodipy® (Invitrogen)
Cy2®, Cy3®, Cy5®, Cy5.5®, Cy7® and Cy7.5® (GE Healthcare)
DyLight™ (ThermoFisher)
IRDye® (LI-COR)
QSY® (Life Technologies)
QXL™ (AnaSpec)
Texas Red® (Invitrogen)

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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 Peptides

2

Dye-labeled peptides are important tools in biochemical and cellular studies. Fluorescent peptides have been extensively used in fluorescence fluorimetry, fluorescence microscopy, fluorescence polarization spectroscopy, time-resolved fluorescence (TRF) and fluorescence resonance energy transfer (FRET). Fluorescent peptides participating in peptide-receptor interactions can be monitored to determine the location of receptors in cells or tissues, to allow quantification of receptors, to determine receptor affinity for various unknown ligands (drug screening), and to identify various populations of cells endowed with peptide receptors. FRET peptides are widely used for detecting the activities of proteases and protein kinases. Other applications include receptor sorting using fluorescence-associated cell sorting and the measurement of serum peptide levels using fluorescent immunoassays either *in vivo* or *in vitro* for research and diagnostic purposes. The most important characteristics of fluorescent peptides are high sensitivity and non-radioactive detection.

2.1 Selection of a Fluorescent Dye for Labeling Peptides

A fluorescent dye can be attached to a peptide at a specific point through a covalent bond depending on the sequence of peptide. The linkage between dye and peptide 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 peptide to minimize the alteration of peptide biological activity. For all the peptide labelings, the dye need be attached at a defined position: N-terminus, C-terminus, 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 reagents for facilitating the conjugation of dyes to peptides that are used for a variety of biological studies.

2.2 Coumarin Dyes

Coumarin dyes are predominantly used for preparing blue fluorescent peptides. Among them, 7-hydroxy, 7-alkoxy and 7-aminocoumarin dyes are more frequently used. AMCA might be the best blue fluorescent dye for labeling peptides due to its high fluorescence quantum yield and pH-insensitivity. Our Tide Flour™ 1 (TF1) is a water-soluble derivative of AMCA, and can be used for improving peptide water solubility if the labeling of a peptide by AMCA significantly decreases its water solubility. AMCA and TF1 have almost identical spectral properties. Besides AMCA and TF1, AAT Bioquest also offers other coumarin dyes for labeling peptides.

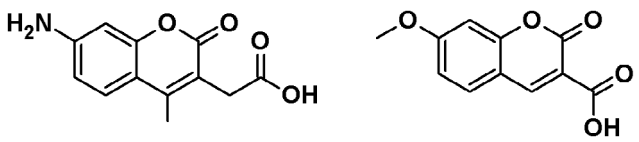

Figure 2.1. The chemical structures of AMCA acid (Cat# 501, left) and 7-methoxycoumarin-3-carboxylic acid (Cat# 560, right), the two most common coumarin dyes for labeling peptides.

Table 2.1 Coumarin Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
501	AMCA Acid	25 mg	353	455	18,000	0.153
507	AMCA Alkyne	1 mg	353	455	19,000	0.153
508	AMCA Azide	1 mg	353	455	19,000	0.153
503	AMCA C2 Maleimide	5 mg	353	455	19,000	0.153
504	AMCA Ethylenediamine	5 mg	353	455	19,000	0.153
502	AMCA, Succinimidyl Ester	10 mg	353	455	19,000	0.153
505	DEAC [7-Diethylaminocoumarin-3-carboxylic acid]	100 mg	427	478	40,000	Not Determined
506	DEAC, SE [7-Diethylaminocoumarin-3-carboxylic acid, Succinimidyl Ester]	25 mg	427	478	40,000	Not Determined
554	7-Hydroxy-4-methylcoumarin-3-acetic Acid	100 mg	360	455	20,000	0.100
556	7-Hydroxy-4-methylcoumarin-3-acetic Acid, Succinimidyl Ester	25 mg	364	458	25,000	0.100
550	7-Hydroxycoumarin-3-carboxylic Acid	250 mg	387	448	25,000	0.0815
551	7-Hydroxycoumarin-3-carboxylic Acid, Succinimidyl Ester	50 mg	419	447	35,000	0.0815
552	7-Hydroxycoumarin-4-acetic Acid	100 mg	360	450	18,000	0.100
553	7-Hydroxycoumarin-4-acetic Acid, Succinimidyl Ester	25 mg	360	450	18,000	0.100
557	MCA [7-Methoxycoumarin-4-acetic Acid]	1 g	322	390	15,000	0.300
558	MCA Succinimidyl Ester [7-Methoxycoumarin-4-acetic Acid, Succinimidyl Ester]	25 mg	322	390	15,000	0.300
560	7-Methoxycoumarin-3-carboxylic Acid	1 g	336	402	20,000	0.171
563	7-Methoxycoumarin-3-carboxylic Acid, Succinimidyl Ester	100 mg	358	410	25,000	0.171

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2.3 Fluorescein Dyes

Fluorescein derivatives are the most common fluorescent derivatization reagents for covalently labeling peptides. In addition to their high absorptivity, excellent fluorescence quantum yields and good water solubility, fluorescein derivatives 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. 5-FAM derivatives have been predominantly used for labeling peptides. AAT Bioquest offers the most complete product line of FAM for labeling peptides.

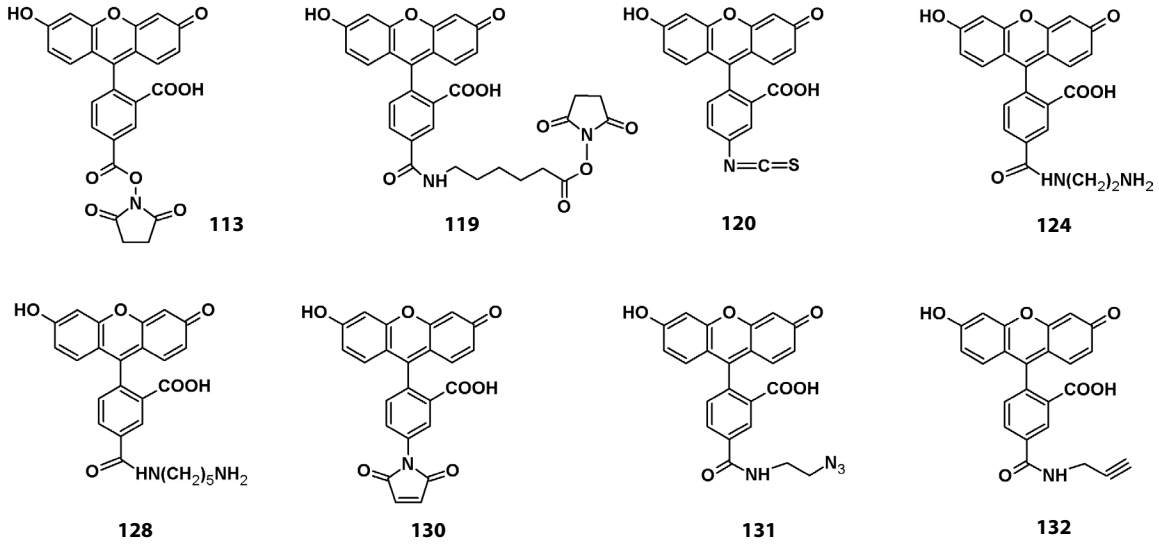

Figure 2.2. The chemical structures of typical fluorescein dyes for labeling peptides

Table 2.2 Fluorescein Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
103	5-FAM [5-Carboxyfluorescein] *Single Isomer*	100 mg	494	521	75,000	0.178
105	5-FAM [5-Carboxyfluorescein] *Validated for Labeling Peptides*	5 g	494	521	75,000	0.178
113	5-FAM, SE [5-Carboxyfluorescein, Succinimidyl Ester] *Single Isomer*	10 mg	494	521	75,000	0.178
115	5-FAM, SE [5-Carboxyfluorescein, Succinimidyl Ester] *Validated for Labeling Peptides*	1 g	494	521	75,000	0.178
132	5-FAM Alkyne	10 mg	494	521	75,000	0.178
131	5-FAM Azide	10 mg	494	521	75,000	0.178
128	5-FAM Cadaverine	100 mg	494	521	75,000	0.178
124	5-FAM Ethylenediamine	100 mg	494	521	75,000	0.178
119	5-FAM-X, SE	5 mg	494	521	75,000	0.178
100	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Mixed Isomers*	1 g	494	519	75,000	0.172
101	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for Labeling Peptides and Oligos*	10 g	494	519	75,000	0.172
110	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Mixed Isomers*	25 mg	494	519	75,000	0.172
112	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Validated for Labeling Peptides and Oligos*	1 g	494	519	75,000	0.172
127	5(6)-FAM Cadaverine	100 mg	494	519	75,000	0.172
123	5(6)-FAM Ethylenediamine	100 mg	494	519	75,000	0.172
120	5-FITC [FITC Isomer I; Fluorescein-5-isothiocyanate] *UltraPure Grade*	100 mg	492	515	76,000	0.254
121	5-FITC [FITC Isomer I; Fluorescein-5-isothiocyanate] *UltraPure Grade*	1 g	492	515	76,000	0.254
130	Fluorescein-5-maleimide	25 mg	491	514	74,000	0.275

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 much higher photostability than fluoresceins and coumarins. Carboxytetramethylrhodamine (TAMRA) is a common fluorophore for preparing peptide conjugates. Sulforhodamine 101 sulfonyl chloride (Texas Red®) is another popular peptide 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 9).

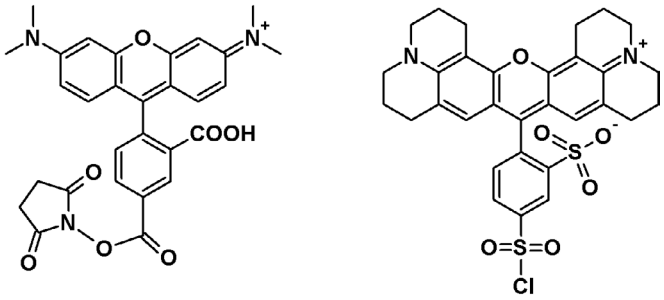

Figure 2.3. The chemical structures of 5-TAMRA, SE (Cat# 373, left) and Texas Red® (Cat# 480, right).

Table 2.3 Rhodamine Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.360
322	5-CR110 [5-Carboxyrhodamine 110] *Single Isomer*	5 mg	498	521	75,000	0.091
351	5-CR110, SE [5-Carboxyrhodamine 110, Succinimidyl Ester] *Single Isomer*	5 mg	498	521	80,000	0.091
331	5-CR6G [5-Carboxyrhodamine 6G] *Single Isomer*	10 mg	518	544	80,000	0.214
341	5-CR6G, SE [5-Carboxyrhodamine 6G, Succinimidyl Ester] *Single Isomer*	1 mg	524	556	80,000	0.214
320	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed Isomers*	100 mg	498	521	75,000	0.091
350	5(6)-CR110, SE [5-(and 6)-Carboxyrhodamine 110, succinimidyl ester] *Mixed isomers*	5 mg	498	521	78,000	0.091
330	5(6)-CR6G [5-(and 6)-Carboxyrhodamine 6G]	25 mg	519	544	80,000	0.214
340	5(6)-CR6G, SE [5-(and 6)-Carboxyrhodamine 6G, Succinimidyl Ester] *Mixed Isomers*	10 mg	522	550	86,000	0.214
470	Lissamine Rhodamine B Sulfonyl Chloride [Sulforhodamine B Sulfonyl Chloride]	100 mg	568	583	85,000	0.171
381	5-ROX [5-Carboxy-X-rhodamine] *Single Isomer*	5 g	567	591	86,000	0.168
391	5-ROX, SE [5-Carboxy-X-rhodamine, Succinimidyl Ester] *Single Isomer*	100 mg	573	602	95,000	0.168
380	5(6)-ROX [5-(and 6)-Carboxy-X-rhodamine] *Mixed Isomers*	100 mg	568	591	85,000	0.168
390	5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, Succinimidyl Ester]	25 mg	576	601	93,000	0.168
480	Sulforhodamine 101 Sulfonyl Chloride [Also known as *Texas Red®*]	10 mg	588	601	100,000	0.360
363	5-TAMRA [5-Carboxytetramethylrhodamine] *Single Isomer*	10 mg	541	568	75,000	0.178
365	5-TAMRA [5-Carboxytetramethylrhodamine] *Validated for Labeling Peptides*	1 g	541	568	75,000	0.178
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester] *Single Isomer*	5 mg	547	575	78,000	0.178
375	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester] *Validated for Labeling Peptides*	1 g	547	575	78,000	0.178
360	5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed Isomers*	100 mg	541	565	75,000	0.187
362	5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed Isomers*	5 g	541	565	75,000	0.187
370	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] *Mixed Isomers*	25 mg	546	575	77,000	0.187
372	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] *Mixed Isomers*	1 g	546	575	77,000	0.187
485	Texas Red® Alkyne [TR Alkyne]*Single Isomer*	5 mg	588	601	95,000	0.360
484	Texas Red® Azide [TR Azide]*Single Isomer*	5 mg	588	601	95,000	0.360

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

Although sulforhodamine 101 acid chloride (also called Texas Red®) is the most popular labeling reagent of sulfonyl chloride, it is quite unstable in water, especially at the higher pH required for reaction 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, peptides 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 peptides. 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 peptides that are often poorly labeled by Texas Red® or Texas Red®-X. The conjugates of hydrophobic peptides 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 almost identical to those of Texas Red®*
- *Fluorescence less-quenched on proteins than Texas Red®*
- *More stable than Texas Red®*
- *Higher conjugation yield*

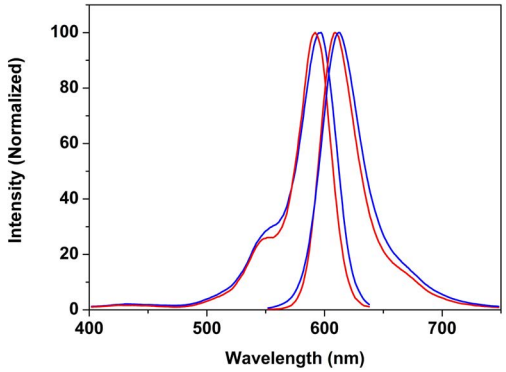

Figure 2.4. Spectral comparison of California Red™ and Texas Red® conjugated to Gly-Gly-Sor-Ser-Arg-Trp (Red: Texas Red®; Blue: California Red™).

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	NHS Ester	NHS Ester	Sulfonyl Chloride
Water Solubility (pH 7.0)	<1 mg/mL	>10 mg/mL	<1 mg/mL
Conjugation Yield (after HPLC Purification)**	76%	71%	26%

* Glycine conjugate; ** Based on the reaction with Gly-Gly-Ser-Ser-Arg-Trp.

Table 2.5 Superior Replacement Dyes for Texas Red®

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.360
480	Sulforhodamine 101 Sulfonyl Chloride [also known as Texas Red®]	10 mg	588	601	100,000	0.360
472	SunRed™, SE	5 mg	583	603	98,000	0.366
485	Texas Red® Alkyne *Single Isomer*	5 mg	588	601	95,000	0.360
484	Texas Red® Azide *Single Isomer*	5 mg	588	601	95,000	0.360
482	Texas Red® Cadaverine *Single Isomer*	5 mg	582	602	95,000	0.360
481	Texas Red® Hydrazide *Single Isomer*	5 mg	582	602	95,000	0.360
483	Texas Red® Maleimide *Single Isomer*	5 mg	588	601	95,000	0.360

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 IR 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 2.6 on page 11) in the literature. However, some vendors offer the less expensive non-sulfonated cyanines (Figure 2.5) 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 yield 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.

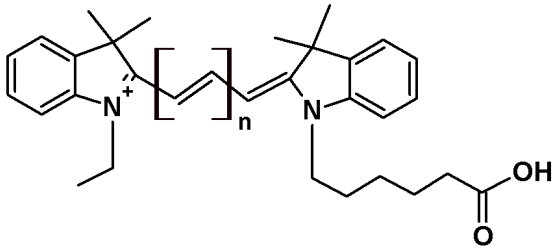

Figure 2.5a. The chemical structures of typical non-sulfonated cyanine dyes for labeling peptides (Cy3NS: n=1; Cy5NS: n=2; Cy7NS: n=3).

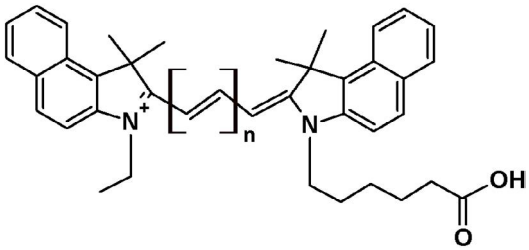

Figure 2.5b. The chemical structures of typical non-sulfonated cyanine dyes for labeling peptides (Cy3.5NS: n=1; Cy5.5NS: n=2; Cy7.5NS: n=3).

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 Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
190	Cy3NS Acid	100 mg	549	565	145,000	0.073
191	Cy3NS, Succinimidyl Ester	25 mg	549	565	145,000	0.073
194	Cy5NS Acid	100 mg	644	665	230,000	0.030
195	Cy5NS, Succinimidyl Ester	25 mg	644	665	230,000	0.030
197	Cy7NS Acid	100 mg	750	780	250,000	0.036
198	Cy7NS, Succinimidyl Ester	25 mg	750	780	250,000	0.036
181	ICG-ATT [3-ICG-acyl-1,3-thiazolidine-2-thione]	1 mg	780	800	240,000	0.076
182	ICG-OSu	1 mg	780	800	240,000	0.076
180	ICG-Sulfo-OSu	1 mg	780	800	240,000	0.076

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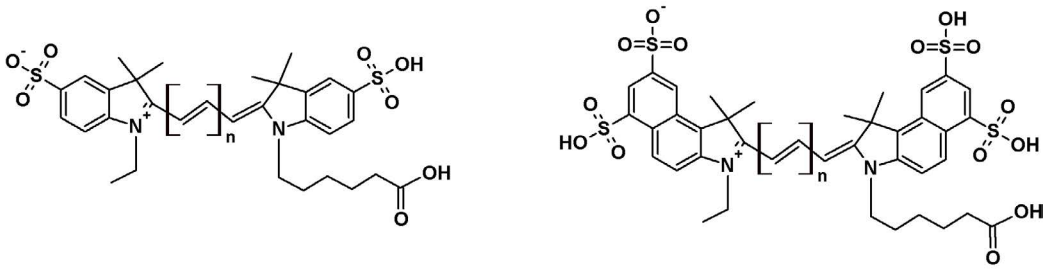

Figure 2.6. The chemical structures of sulfonated cyanine dyes for labeling peptides. (Left: Cy3: n=1; Cy5: n=2; Cy7: n=3. Right: Cy3.5: n=1; Cy5.5: n= 2; Cy7.5: n=3).

Table 2.8 Sulfonated Cyanine Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
144	Cyanine 3 Alkyne [equivalent to Cy3® Alkyne]	1 mg	555	565	150,000	0.073
145	Cyanine 3 Amine [equivalent to Cy3® Amine]	1 mg	555	565	150,000	0.073
143	Cyanine 3 Azide [equivalent to Cy3® Azide]	1 mg	555	565	150,000	0.073
146	Cyanine 3 Hydrazide [equivalent to Cy3® Hydrazide]	1 mg	555	565	150,000	0.073
142	Cyanine 3 Maleimide [equivalent to Cy3® Maleimide]	1 mg	555	565	150,000	0.073
140	Cyanine 3 Monoacid [equivalent to Cy3® Acid]	5 mg	555	565	150,000	0.073
141	Cyanine 3, Monosuccinimidyl Ester [equivalent to Cy3® NHS Ester]	1 mg	555	565	150,000	0.073
139	Cyanine 3.5 Amine [equivalent to Cy3.5® Amine]	1 mg	581	596	125,000	0.178
149	Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide]	1 mg	581	596	125,000	0.178
148	Cyanine 3.5, Monosuccinimidyl Ester [equivalent to Cy3.5® NHS Ester]	1 mg	581	596	125,000	0.178
154	Cyanine 5 Alkyne [equivalent to Cy5® Alkyne]	1 mg	649	665	250,000	0.030
155	Cyanine 5 Amine [equivalent to Cy5® Amine]	1 mg	649	665	250,000	0.030
153	Cyanine 5 Azide [equivalent to Cy5® Azide]	1 mg	649	665	250,000	0.030
156	Cyanine 5 Hydrazide [equivalent to Cy5® Hydrazide]	1 mg	649	665	250,000	0.030
152	Cyanine 5 Maleimide [equivalent to Cy5® Maleimide]	1 mg	649	665	250,000	0.030
150	Cyanine 5 Monoacid [equivalent to Cy5® Acid]	5 mg	649	665	250,000	0.030
151	Cyanine 5, Monosuccinimidyl Ester [equivalent to Cy5® NHS Ester]	1 mg	649	665	250,000	0.030
179	Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne]	1 mg	678	701	230,000	0.101
176	Cyanine 5.5 Amine [equivalent to Cy5.5® Amine]	1 mg	678	701	230,000	0.101
178	Cyanine 5.5 Azide [equivalent to Cy5.5® Azide]	1 mg	678	701	230,000	0.101
177	Cyanine 5.5 Hydrazide [equivalent to Cy5.5® Hydrazide]	1 mg	678	701	230,000	0.101
175	Cyanine 5.5 Maleimide [equivalent to Cy5.5® Maleimide]	1 mg	678	701	230,000	0.101
173	Cyanine 5.5 Monoacid [equivalent to Cy5.5® Acid]	5 mg	678	701	230,000	0.101
174	Cyanine 5.5, Monosuccinimidyl Ester [equivalent to Cy5.5® NHS Ester]	1 mg	678	701	230,000	0.101
164	Cyanine 7 Alkyne [equivalent to Cy7® Alkyne]	1 mg	749	776	275,000	0.036
165	Cyanine 7 Amine [equivalent to Cy7® Amine]	1 mg	749	776	275,000	0.036
163	Cyanine 7 Azide [equivalent to Cy7® Azide]	1 mg	749	776	275,000	0.036
166	Cyanine 7 Hydrazide [equivalent to Cy7® Hydrazide]	1 mg	749	776	275,000	0.036
162	Cyanine 7 Maleimide [equivalent to Cy7® Maleimide]	1 mg	749	776	275,000	0.036
160	Cyanine 7 Monoacid [equivalent to Cy7® Acid]	5 mg	749	776	275,000	0.036
161	Cyanine 7, Monosuccinimidyl Ester [equivalent to Cy7® NHS Ester]	1 mg	749	776	275,000	0.036

2.7 Tide Fluor™ Dyes Optimized for Labeling Peptides

Although EDANS, FAM, TAMRA, ROX, Cy3® and Cy5® have been widely used to develop a variety of peptide 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 peptide 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 peptide 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 peptides, 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 2.7).

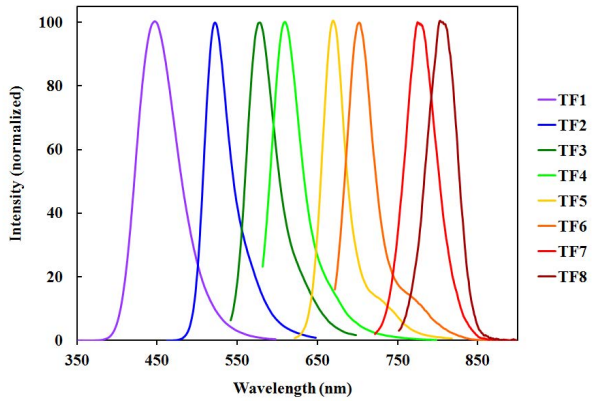

Figure 2.7. The normalized fluorescence spectra of Tide Fluor™ dyes.

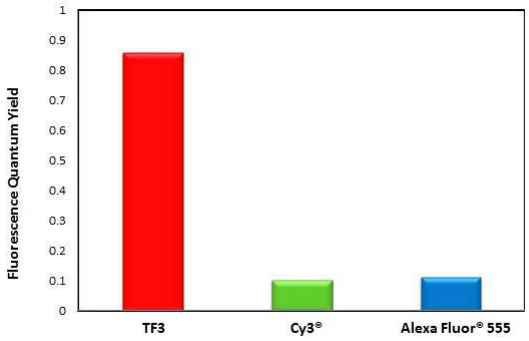

Figure 2.8. Fluorescence quantum yield comparison of TF3, Cy3® and Alexa Fluor® 555 on the peptide of Dye-PLSRTLSVAAKK-NH_2.

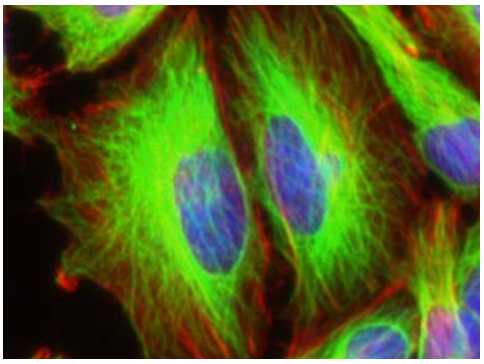

Figure 2.9. Image of HeLa cells. Tublins were stained with mouse anti-tubulin followed with iFluor™ 488 Goat Anti-Mouse IgG (green, Cat# 16448), actin filaments were stained with TF3- Phalloidin Conjugate (red, Cat# 23119), and nuclei were stained with Hoechst 33342 (blue).

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

Table 2.11 Tide Fluor™ Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
2238	Tide Fluor™ 1 Acid [TF1 Acid] *Superior Replacement for EDANS*	100 mg	345	442	20,000	0.187
2237	Tide Fluor™ 1 Alkyne [TF1 Alkyne] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2239	Tide Fluor™ 1 Amine [TF1 Amine] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2236	Tide Fluor™ 1 Azide [TF1 Azide] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2348	Tide Fluor™ 2WS Acid [TF2WS Acid] *Superior Replacement for FITC*	10 mg	502	525	75,000	0.091
2349	Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC*	5 mg	502	525	75,000	0.091
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	0.436
2301	Tide Fluor™ 4 Alkyne [TF4 Alkyne] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2286	Tide Fluor™ 4 Amine [TF4 Amine] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2300	Tide Fluor™ 4 Azide [TF4 Azide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2287	Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2289	Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®*	5 mg	590	618	90,000	0.436
2278	Tide Fluor™ 5WS Acid [TF5WS Acid] *Superior Replacement for Cy5®*	10 mg	649	664	250,000	0.027
2276	Tide Fluor™ 5WS Alkyne [TF5WS Alkyne] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2279	Tide Fluor™ 5WS Amine [TF5WS Amine] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2275	Tide Fluor™ 5WS Azide [TF5WS Azide] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2280	Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2281	Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®*	5 mg	649	664	250,000	0.027
2291	Tide Fluor™ 6WS Acid [TF6WS Acid] *Superior Replacement for Cy5.5®*	10 mg	676	695	220,000	0.101
2303	Tide Fluor™ 6WS Alkyne [TF6WS Alkyne] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2292	Tide Fluor™ 6WS Amine [TF6WS Amine] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2302	Tide Fluor™ 6WS Azide [TF6WS Azide] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2293	Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2294	Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2330	Tide Fluor™ 7WS Acid [TF7WS Acid] *Superior Replacement for Cy7®*	10 mg	749	775	275,000	0.049
2305	Tide Fluor™ 7WS Alkyne [TF7WS Alkyne] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2331	Tide Fluor™ 7WS Amine [TF7WS Amine] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2304	Tide Fluor™ 7WS Azide [TF7WS Azide] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2332	Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2333	Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2335	Tide Fluor™ 8WS Acid [TF8WS Acid] *Near Infrared Emission*	10 mg	775	807	250,000	0.109
2307	Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2336	Tide Fluor™ 8WS Amine [TF8WS Amine] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2306	Tide Fluor™ 8WS Azide [TF8WS Azide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2337	Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2338	Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission*	1 mg	775	807	250,000	0.109

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2.8 Other Dyes

EDANS dyes are quite useful for preparing fluorescent peptides. They might be the first group of fluorescent dyes used for preparing FRET peptides to analyze a broad range of protease activities. AAT Bioquest offers a variety of EDANS derivatives for preparing FRET peptide substrates.

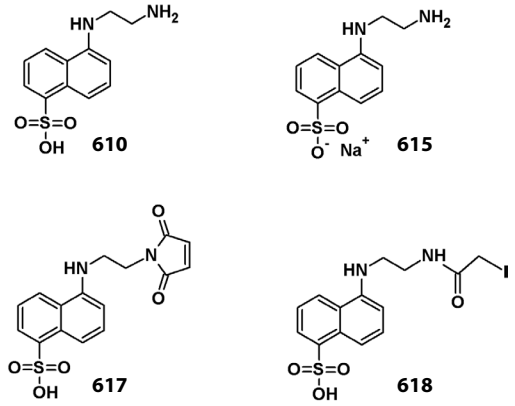

Figure 2.10. The chemical structures of EDANS dyes.

Besides the popular fluorescent dyes such as coumarins, fluoresceins, rhodamines, cyanines and EDANS dyes, AAT Bioquest also offers some fluorescent dyes that are ocassionally used for labeling peptides. Our Bodi Fluor™ dyes are chemically the same as the Bodipy® dyes offered by Invitrogen, e.g., Bodi Fluor™ 488 is equivalent to Bodipy® FL. Bodi Fluor™ 488 is highly fluorescent, and often used as an alternative to fluorescein since its fluorescence is not pH-dependent. The sharper excitation and emission peaks of Bodi Fluor™ 488 make it better suitable for multi-color fluorescence imaging applications.

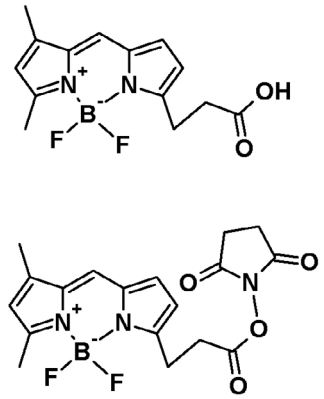

Figure 2.11. The chemical structures of Bodi Fluor™ 488 acid (Cat# 700, top) and its succinimidyl ester (Cat# 701, bottom).

Dansyl dyes are usually used to label peptides for biophysical investigations due to their small sizes and little interference to the binding affinity of peptides. The environmental sensitivity of Dansyl dyes is often used to probe biological microenvironments.

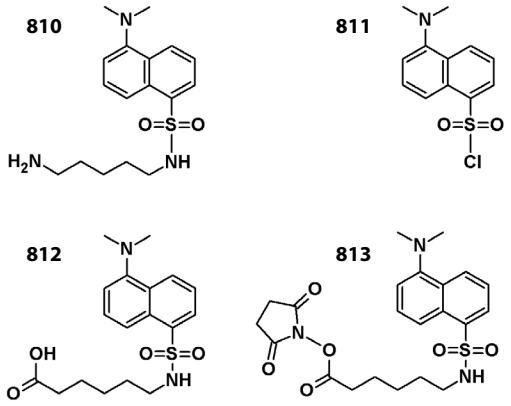

Figure 2.12. The chemical structures of typical Dansyl dyes.

Table 2.12 Bodi Fluor™, Dansyl and EDANS Derivatives for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @280 nm
700	Bodi Fluor™ 488 Acid	10 mg	505	512	70,000	0.018
701	Bodi Fluor™ 488, SE	5 mg	505	511	70,000	0.018
810	Dansyl Cadaverine	25 mg	333	518	4,000	0.387
811	Dansyl Chloride	100 mg	335	518	4,000	0.387
812	Dansyl-X Acid	5 g	335	518	4,000	0.387
813	Dansyl-X, SE	1 g	335	518	4,000	0.387
610	EDANS Acid	1 g	335	493	5,500	0.107
617	EDANS C2 Maleimide	25 mg	335	493	5,500	0.107
618	EDANS Iodoacetamide	25 mg	335	493	5,500	0.107
615	EDANS, Sodium Salt	1 g	335	493	5,500	0.107

3 Non-Fluorescent Quenchers for Labeling Peptides

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3.1 FRET Peptide Substrates

FRET is a physical phenomenon, that is being used more and more in biomedical research and drug discovery today. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. The donor molecule is the dye or chromophore that initially absorbs the energy and the acceptor is the chromophore to which the energy is subsequently transferred. This resonance interaction occurs over greater than interatomic distances, without conversion to thermal energy and without any molecular collision. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime, and an increase in the acceptor's emission intensity. A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor/acceptor pair. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions.

While there are many factors that influence FRET, the primary conditions that need to be met in order for FRET to occur are relatively few. The donor and acceptor molecules must be in close proximity to one another (typically 10-100 Å). The absorption or excitation spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor. The degree to which they overlap is referred to as the spectral overlap integral (J). The donor and acceptor transition dipole orientations must be approximately parallel. Assuming that the donor acceptor pairs are compatible, the most critical element necessary for FRET to occur is close proximity of the pairs. Förster, demonstrated that the efficiency of the process (E) depends on the inverse sixth power distance between donor and acceptor as expressed with the following equation:

$$E = R_o^6/(R_o^6 + r^6)$$

Where R_o is the Förster distance at which half the energy is transferred and r is the actual distance between donor and acceptor. The magnitude of the R_o is dependent on the spectral properties of the donor and the acceptor. Förster distances ranging from 20 to 90 Å are most useful for the studies of biological macromolecules.

Proteases are involved in a number of physiological processes. The detection of proteases and the screening of specific protease inhibitors are essential in the discovery of potential drugs for the treatment and management of protease-related diseases. A variety of donor and acceptor molecules (either fluorescent or non-fluorescent) are attached to the peptide/protein (forming the internally quenched conjugates), and are used to detect a protease depending on the sequences of the amino acids. The donor and acceptor molecules are carefully chosen so that the absorption of the acceptor overlaps with the fluorescence of the donor, thus ensuring that the fluorescence is quenched through resonance energy transfer. Enzyme hydrolysis of the substrate results in spatial separation of the donor and the acceptor molecules, thereby restoring the donor's fluorescence.

3.2 Non-Fluorescent DABCYL Dyes

DABCYL dyes are quite useful for preparing FRET peptides. AAT Bioquest offers a variety of DABCYL derivatives for developing FRET peptide 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 peptides to get superior performance.

Table 3.1 DABCYL Derivatives for Labeling Peptides

Cat. #	Product Name	Size	Ab (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
2001	DABCYL Acid	5 g	426	20,000	0.516
2006	DABCYL C2 Amine	100 mg	428	20,000	0.516
2008	DABCYL C2 Maleimide	25 mg	428	20,000	0.516
2004	DABCYL, Succinimidyl Ester	1 g	453	20,000	0.516
2005	DABCYL, Succinimidyl Ester	5 g	453	20,000	0.516

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.

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.*

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.

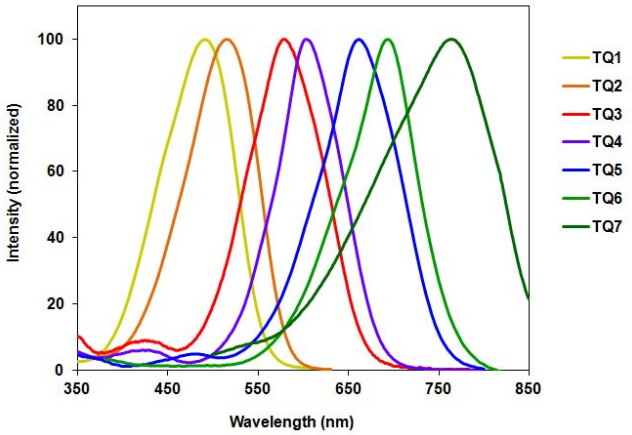

Figure 3.1. The normalized absorption spectra of TQ1, 2, 3, 4, 5, 6 and 7.

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]

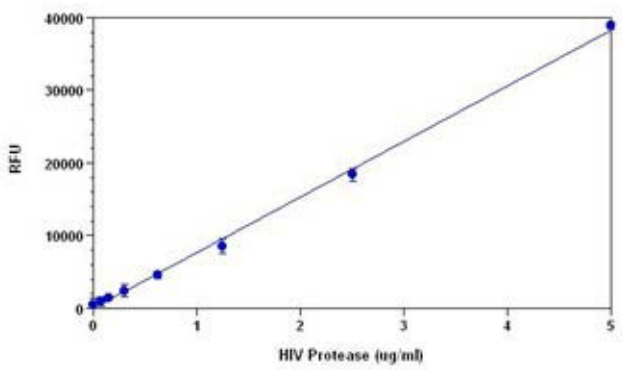

Figure 3.2. HIV Protease cleavage of Arg-Glu(5-FAM)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg. Upon HIV protease cleavage, the fluorescence of 5-FAM is recovered and monitored at Ex/Em = 490 /520 nm.

Table 3.3. Recommended FRET Pairs for Developing Molecular Beacon Nucleic Acid Detection and Protease Assays*

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.

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Table 3.4 Tide Quencher™ Dyes for Developing FRET Protease Assays

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

Table 3.5 Tide Quencher™ Dyes for Labeling Peptides

Cat. #	Product Name	Size	Ab	EC ($cm^{-1} M^{-1}$)	CF @280 nm
2189	Tide Quencher™ 1 Alkyne [TQ1 Alkyne]	5 mg	490	20,000	0.194
2192	Tide Quencher™ 1 Amine [TQ1 Amine]	5 mg	490	20,000	0.194
2188	Tide Quencher™ 1 Azide [TQ1 Azide]	5 mg	490	20,000	0.194
2196	Tide Quencher™ 1 Maleimide [TQ1 Maleimide]	5 mg	490	20,000	0.194
2199	Tide Quencher™ 1, Succinimidyl Ester [TQ1, SE]	25 mg	490	20,000	0.194
2200	Tide Quencher™ 2 Acid [TQ2 Acid]	100 mg	515	21,000	0.120
2212	Tide Quencher™ 2 Alkyne [TQ2 Alkyne]	100 mg	515	21,000	0.120
2202	Tide Quencher™ 2 Amine [TQ2 Amine]	5 mg	515	21,000	0.120
2211	Tide Quencher™ 2 Azide [TQ2 Azide]	5 mg	515	21,000	0.120
2206	Tide Quencher™ 2 Maleimide [TQ2 Maleimide]	5 mg	515	21,000	0.120
2210	Tide Quencher™ 2, Succinimidyl Ester [TQ2, SE]	25 mg	515	21,000	0.120
2058	Tide Quencher™ 2WS, Succinimidyl Ester [TQ2WS, SE]	5 mg	515	19,000	0.559
2220	Tide Quencher™ 3 Acid [TQ3 Acid]	100 mg	570	22,000	0.091
2232	Tide Quencher™ 3 Alkyne [TQ3 Alkyne]	5 mg	570	22,000	0.091
2222	Tide Quencher™ 3 Amine [TQ3 Amine]	5 mg	570	22,000	0.091
2231	Tide Quencher™ 3 Azide [TQ3 Azide]	5 mg	570	22,000	0.091
2226	Tide Quencher™ 3 Maleimide [TQ3 Maleimide]	5 mg	570	22,000	0.091
2230	Tide Quencher™ 3, Succinimidyl Ester [TQ3, SE]	25 mg	570	22,000	0.091
2229	Tide Quencher™ 3WS, Succinimidyl Ester [TQ3WS, SE]	1 mg	578	90,000	0.205
2060	Tide Quencher™ 4WS Acid [TQ4WS Acid]	5 mg	603	90,000	0.136
2069	Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne]	1 mg	603	90,000	0.136
2061	Tide Quencher™ 4WS Amine [TQ4WS Amine]	1 mg	603	90,000	0.136
2068	Tide Quencher™ 4WS Azide [TQ4WS Azide]	1 mg	603	90,000	0.136
2064	Tide Quencher™ 4WS Maleimide [TQ4WS Maleimide]	1 mg	603	90,000	0.136
2067	Tide Quencher™ 4WS, Succinimidyl Ester [TQ4WS, SE]	1 mg	603	90,000	0.136
2083	Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne]	1 mg	661	130,000	0.082
2076	Tide Quencher™ 5WS Amine [TQ5WS Amine]	1 mg	661	130,000	0.082
2082	Tide Quencher™ 5WS Azide [TQ5WS Azide]	1 mg	661	130,000	0.082
2079	Tide Quencher™ 5WS Maleimide [TQ5WS Maleimide]	1 mg	661	130,000	0.082
2081	Tide Quencher™ 5WS, Succinimidyl Ester [TQ5WS, SE]	1 mg	661	130,000	0.082
2090	Tide Quencher™ 6WS Acid [TQ6WS Acid]	5 mg	704	130,000	0.102
2098	Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne]	1 mg	704	130,000	0.102
2091	Tide Quencher™ 6WS Amine [TQ6WS Amine]	1 mg	704	130,000	0.102
2097	Tide Quencher™ 6WS Azide [TQ6WS Azide]	1 mg	704	130,000	0.102
2094	Tide Quencher™ 6WS Maleimide [TQ6WS Maleimide]	1 mg	704	130,000	0.102
2096	Tide Quencher™ 6WS, Succinimidyl Ester [TQ6WS, SE]	1 mg	704	130,000	0.102
2113	Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne]	1 mg	763	140,000	0.091
2106	Tide Quencher™ 7WS Amine [TQ7WS Amine]	1 mg	763	140,000	0.091
2112	Tide Quencher™ 7WS Azide [TQ7WS Azide]	1 mg	763	140,000	0.091
2109	Tide Quencher™ 7WS Maleimide [TQ7WS Maleimide]	1 mg	763	140,000	0.091
2111	Tide Quencher™ 7WS, Succinimidyl Ester [TQ7WS, SE]	1 mg	763	140,000	0.091

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Label N-Terminal Amino Acid & Lysine Residues Using Amine-Reactive Dyes

4

Amine-reactive fluorescent probes are widely used to modify peptides at the N-terminal amino acid or lysine residues. A number of fluorescent amino-reactive dyes have been developed to label various peptides, and the resultant conjugates are widely used in biological applications. Three major classes of amine-reactive fluorescent reagents are currently used to label peptides: succinimidyl esters (SE), isothiocyanates and sulfonyl chlorides. AAT Bioquest offers all the popular amine-reactive fluorescent dyes for labeling peptides. Although FITC (fluorescein isothiocyanate), one of the most popular fluorescent labeling dyes, is predominantly used for preparing a variety of fluorescent bioconjugates, the low conjugation efficiency and short shelf lifetime of its conjugates are still troublesome for some critical biological applications. We strongly recommend that you choose succinimidyl esters if other conditions and factors are equal.

4.1 Fluorescent Dye Carboxylic Acids & Their Succinimidyl Esters

Succinimidyl esters (SE, also called NHS esters) are proven to be the best reagents for amine modifications because the amide bonds formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. There are a few factors that need be considered when SE compounds are used for conjugation reactions: 1). Solvents: For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO). 2). Reaction pH: The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas isothiocyanates may require pH 9.0-10.0 for optimal conjugations. 3). Reaction Buffers: Buffers that contain free amines such as Tris, glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed (such as dialysis) before performing dye conjugations. 4). 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 a peptide with a dye NHS ester at N-terminal amino acid or lysine residues.

Table 4.1 Fluorescent Dye Carboxylic Acids & Their Succinimidyl Esters for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.360
322	5-CR110 [5-Carboxyrhodamine 110] *Single Isomer*	5 mg	498	521	75,000	0.091
331	5-CR6G [5-Carboxyrhodamine 6G] *Single Isomer*	10 mg	518	544	80,000	0.214
341	5-CR6G, SE [5-Carboxyrhodamine 6G, Succinimidyl Ester] *Single Isomer*	1 mg	524	556	80,000	0.214
320	5(6)-CR110 [5-(and 6)-Carboxyrhodamine 110] *Mixed Isomers*	100 mg	498	521	75,000	0.091
350	5(6)-CR110, SE [5-(and 6)-Carboxyrhodamine 110, Succinimidyl Ester]	5 mg	498	521	75,000	0.091
330	5(6)-CR6G [5-(and 6)-Carboxyrhodamine 6G]	25 mg	519	544	80,000	0.214
340	5(6)-CR6G, SE [5-(and 6)-Carboxyrhodamine 6G, Succinimidyl Ester] *Mixed Isomers*	10 mg	522	550	80,000	0.214
140	Cyanine 3 Monoacid [equivalent to Cy3® Acid]	5 mg	555	565	150,000	0.073
141	Cyanine 3, Monosuccinimidyl Ester [equivalent to Cy3® NHS Ester]	1 mg	555	565	150,000	0.073
147	Cyanine 3.5 Acid [equivalent to Cy3.5® Acid]	5 mg	581	596	125,000	0.178
148	Cyanine 3.5, Monosuccinimidyl Ester [equivalent to Cy3.5® NHS Ester]	1 mg	581	596	125,000	0.178
150	Cyanine 5 Monoacid [equivalent to Cy5® Acid]	5 mg	649	665	250,000	0.030
151	Cyanine 5, Monosuccinimidyl Ester [equivalent to Cy5® NHS Ester]	1 mg	649	665	250,000	0.030
173	Cyanine 5.5 Monoacid [equivalent to Cy5.5® Acid]	5 mg	678	701	230,000	0.101
174	Cyanine 5.5, Monosuccinimidyl Ester [equivalent to Cy5.5® NHS Ester]	1 mg	678	701	230,000	0.101
160	Cyanine 7 Monoacid [equivalent to Cy7® Acid]	5 mg	749	776	275,000	0.036
161	Cyanine 7, Monosuccinimidyl Ester [equivalent to Cy7® NHS Ester]	1 mg	749	776	275,000	0.036
2001	DABCYL Acid [4-((4-(Dimethylamino)phenyl)azo)benzoic Acid] *UltraPure Grade*	5 g	426	N/A	20,000	0.516
2004	DABCYL, Succinimidyl Ester [4-((4-(Dimethylamino)phenyl)azo)benzoic Acid, Succinimidyl Ester]	1 g	453	N/A	20,000	0.516

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Table 4.1 Fluorescent Dye Carboxylic Acids & Their Succinimidyl Esters for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
505	DEAC [7-Diethylaminocoumarin-3-carboxylic Acid]	100 mg	432	472	40,000	Not Determined
506	DEAC, SE [7-Diethylaminocoumarin-3-carboxylic Acid, Succinimidyl Ester]	25 mg	432	472	40,000	Not Determined
2021	DNP-X Acid, SE [6-(2,4-Dinitrophenyl)aminohexanoic Acid, Succinimidyl Ester]	25 mg	350	N/A	18,000	0.181
104	5-FAM [5-Carboxyfluorescein] *Validated for Labeling Peptides*	1 g	494	521	75,000	0.178
113	5-FAM, SE [5-Carboxyfluorescein, succinimidyl ester] *Single Isomer*	10 mg	494	521	75,000	0.178
100	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Mixed Isomers*	1 g	494	519	75,000	0.172
102	5(6)-FAM [5-(and-6)-Carboxyfluorescein] *Validated for Labeling Peptides and Oligos*	25 g	494	519	75,000	0.172
110	5(6)-FAM, SE [5-(and-6)-Carboxyfluorescein, Succinimidyl Ester] *Mixed Isomers*	25 mg	494	519	75,000	0.172
554	7-Hydroxy-4-methylcoumarin-3-acetic Acid	100 mg	360	455	35,000	0.100
556	7-Hydroxy-4-methylcoumarin-3-acetic Acid, Succinimidyl Ester	25 mg	364	458	35,000	0.100
550	7-Hydroxycoumarin-3-carboxylic Acid	250 mg	387	448	25,000	0.082
551	7-Hydroxycoumarin-3-carboxylic Acid, Succinimidyl Ester	50 mg	363	447	25,000	0.082
560	7-Methoxycoumarin-3-carboxylic Acid	1 g	336	402	20,000	0.171
563	7-Methoxycoumarin-3-carboxylic Acid, Succinimidyl Ester	100 mg	358	410	25,000	0.171
381	5-ROX [5-Carboxy-X-rhodamine] *Single Isomer*	5 g	567	591	86,000	0.168
391	5-ROX, SE [5-Carboxy-X-rhodamine, Succinimidyl Ester] *Single Isomer*	100 mg	573	602	95,000	0.168
380	5(6)-ROX [5-(and 6)-Carboxy-X-rhodamine] *Mixed Isomers*	100 mg	568	591	85,000	0.168
390	5(6)-ROX, SE [5-(and-6)-Carboxy-X-rhodamine, Succinimidyl Ester] *Mixed Isomers*	25 mg	576	601	93,000	0.168
363	5-TAMRA [5-Carboxytetramethylrhodamine] *Single Isomer*	10 mg	541	568	75,000	0.178
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester] *Single Isomer*	5 mg	547	575	78,000	0.178
360	5(6)-TAMRA [5-(and-6)-Carboxytetramethylrhodamine] *Mixed Isomers*	100 mg	541	565	75,000	0.187
370	5(6)-TAMRA, SE [5-(and-6)-Carboxytetramethylrhodamine, Succinimidyl Ester] *Mixed Isomers*	25 mg	546	575	75,000	0.187
2238	Tide Fluor™ 1 Acid [TF1 Acid] *Superior Replacement for EDANS*	100 mg	345	442	20,000	0.187
2244	Tide Fluor™ 1, Succinimidyl Ester [TF1, SE] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2245	Tide Fluor™ 2 Acid [TF2 Acid] *Superior Replacement for Fluorescein*	25 mg	500	527	75,000	0.090
2248	Tide Fluor™ 2, Succinimidyl Ester [TF2, SE] *Superior Replacement for Fluorescein*	5 mg	500	527	75,000	0.090
2348	Tide Fluor™ 2WS Acid [TF2WS Acid] *Superior Replacement for FITC*	10 mg	502	525	75,000	0.091
2349	Tide Fluor™ 2WS, Succinimidyl Ester [TF2WS, SE] *Superior Replacement for FITC*	5 mg	502	525	75,000	0.091
2268	Tide Fluor™ 3 Acid [TF3 Acid] *Superior Replacement for Cy3®*	25 mg	555	584	75,000	0.179
2271	Tide Fluor™ 3, Succinimidyl Ester [TF3, SE] *Superior Replacement for Cy3®*	5 mg	555	584	75,000	0.179
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	0.436
2289	Tide Fluor™ 4, Succinimidyl Ester [TF4, SE] *Superior Replacement for ROX and Texas Red®*	5 mg	590	618	90,000	0.436
2278	Tide Fluor™ 5WS acid [TF5WS acid] *Superior replacement for Cy5®*	10 mg	649	664	250,000	0.027
2281	Tide Fluor™ 5WS, Succinimidyl Ester [TF5WS, SE] *Superior Replacement for Cy5®*	5 mg	649	664	250,000	0.027
2291	Tide Fluor™ 6WS Acid [TF6WS Acid] *Superior Replacement for Cy5.5®*	10 mg	676	695	220,000	0.101
2294	Tide Fluor™ 6WS, Succinimidyl Ester [TF6WS, SE] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2330	Tide Fluor™ 7WS Acid [TF7WS Acid] *Superior Replacement for Cy7®*	10 mg	749	775	275,000	0.049
2333	Tide Fluor™ 7WS, Succinimidyl Ester [TF7WS, SE] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2335	Tide Fluor™ 8WS Acid [TF8WS Acid] *Near Infrared Emission*	10 mg	775	807	250,000	0.109
2338	Tide Fluor™ 8WS, Succinimidyl Ester [TF8WS, SE] *Near Infrared Emission*	1 mg	775	807	250,000	0.109

4.2 Dye Sulfonyl Chlorides

Sulfonyl chlorides (SC) are highly reactive. These reagents are unstable in water, especially at the higher pH required for reaction 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 (including tyrosine), aliphatic alcohols (including polysaccharides), thiols (such as cysteine) and imidazoles (such as histidine), but these reactions are not common in proteins or in aqueous solution. 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. We found that the mixed solvent of DMF/chloroform gives the best reaction yield. SC dyes are first dissolved in chloroform, the chloroform solution is then diluted with DMF.

- **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 pH 9.0-10.0 for optimal conjugations.

- **Reaction Buffers:** Buffers that contain free amines such as Tris and glycine must be avoided when using an amine-reactive reagent. Ammonium sulfate and other amines must be removed before performing dye conjugations.

Although Texas Red® and Lissamine rhodamine B sulfonyl chloride are the most popular labeling reagents of sulfonyl chloride, they are quite unstable in water. Even in anhydrous DMF, they tend to give a very complicated reaction mixture. They react with thiols, alcohols, phenols, aliphatic amines and aromatic amines indiscriminatingly. California Red™ SE is a succinimidyl ester and has the spectral properties identical to those of Texas Red®. It is a superior replacement for Texas Red®. LRB Red™ is also a succinimidyl ester and has the spectral properties identical to those of Lissamine rhodamine B sulfonyl chloride. It is a superior replacement for Lissamine rhodamine B sulfonyl chloride. Both California Red™ and LRB Red™ only react with aliphatic amines such as amino acids, peptides and proteins to give bright red fluorescent conjugates. Compared to Texas Red® and Lissamine rhodamine B sulfonyl chloride, California Red™ and LRB Red™ have much higher labeling efficiency, and more importantly the resulted conjugates are much easier to be purified due to the much cleaner reactions. We strongly recommend that you consider using California Red™ and LRB Red™ to replace Texas Red® and Lissamine rhodamine B sulfonyl chloride.

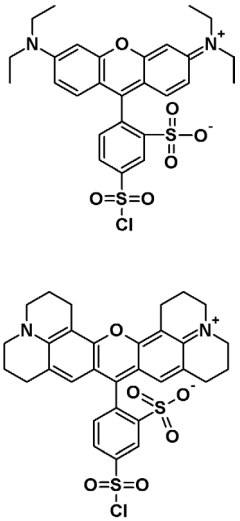

Figure 4.2. The chemical structures of Lissamine rhodamine B sulfonyl chloride (Cat# 470, top) and Texas Red® (Cat# 480, bottom).

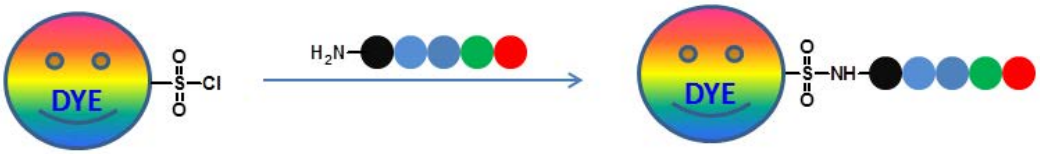

Figure 4.3. The reaction scheme of a peptide with a dye sulfonyl chloride at N-terminal amino acid or lysine residues.

Table 4.2 Dye Sulfonyl Chlorides for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
473	California Red™ SE	5 mg	583	603	100,000	0.360
470	Lissamine Rhodamine B Sulfonyl Chloride	100 mg	568	583	95,000	0.171
471	LRB Red™ SE	10 mg	568	583	95,000	0.171
480	Sulforhodamine 101 Sulfonyl Chloride [Texas Red®]	10 mg	588	601	95,000	0.360
472	SunRed™ SE	5 mg	583	603	98,000	0.366

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4.3 Dye Isothiocyanates

Isothiocyanates form thioureas upon reaction with amines. It is proven that some thiourea products (in particular, the conjugates from α-amino acids/peptides/proteins) are much less stable than the conjugates that are prepared from the corresponding succinimidyl esters (SE). It has been reported that antibody conjugates prepared from fluorescein isothiocyanates deteriorate over time. We strongly recommend that you use succinimidyl esters for your conjugations whenever possible.

There are a few factors that need be considered when isothiocyanate compounds are used for conjugation reaction:

- **Solvents:** For the most part, isothiocyanate reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

- **Reaction pH:** The labeling reactions of amines with isothiocyanates are strongly pH dependent. Isothiocyanate reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the α-amino groups of lysines. Protein modifications by isothiocyanates may require a pH 9.0-10.0 for optimal conjugations.

- **Reaction Buffers:** Buffers that contain free amines such as Tris and glycine must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed before performing dye conjugations. 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:** Isothiocyanate conjugations are usually done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

Although 5-TRITC and 6-TRITC have been used for preparing fluorescent peptides, the conjugation yields are generally lower than the corresponding 5-TAMRA SE and 6-TAMRA SE. In addition, the peptide conjugates of 5-TRITC and 6-TRITC are generally less stable than those of 5-TAMRA SE and 6-TAMRA SE. We have tested a number of fluorescent peptides prepared from TAMRA SE and TRITC. They have almost identical spectral properties and biological activities. Thus we suggest you use 5-TAMRA SE and 6-TAMRA SE to replace 5-TRITC and 6-TRITC for preparing most bioconjugates.

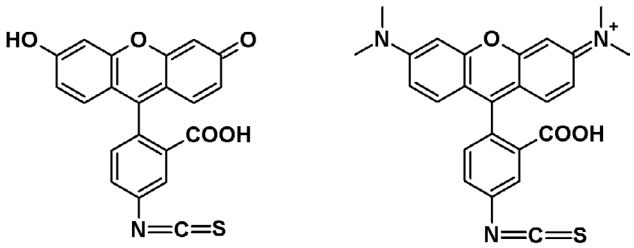

Figure 4.4. The chemical structures of 5-FITC (Cat# 120, left) and 5-TRITC (right).

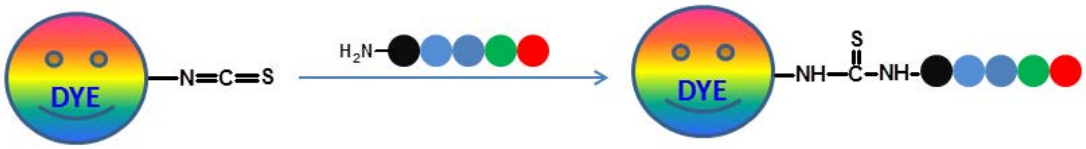

Figure 4.5. The reaction scheme of a peptide with a dye isothiocyanate at N-terminal amino acid or lysine residues.

Table 4.3 Dye Isothiocyanates for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
120	5-FITC [FITC Isomer I; Fluorescein-5-isothiocyanate] *UltraPure Grade*	100 mg	492	515	76,000	0.254
373	5-TAMRA, SE [5-Carboxytetramethylrhodamine, Succinimidyl Ester]	5 mg	547	575	78,000	0.178

5 Label C-Terminal Amino Acid, Asp & Glu Residues Using Amine-Containing Dyes

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Amine-containing dyes are used to modify peptides with water-soluble carbodiimides (such as EDC) to convert the carboxy groups of the peptides into amide groups. Either NHS or NHSS may be used to improve the coupling efficiency of EDC-mediated protein–carboxylic acid conjugations. A large excess of the amine-containing dyes is usually used for EDC-mediated bioconjugations in large concentrated peptide solutions at low pH to reduce intra- and inter-protein coupling to lysine residues, a common side reaction. AAT Bioquest offers the largest collection of fluorescent dyes that contain an aliphatic amino group. The amino group can be coupled to a carbonyl group.

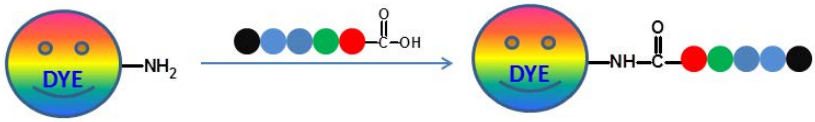

Figure 5.1. The reaction scheme of a peptide with a dye amine at C-terminal amino acid, Asp and Glu residues.

Table 5.1 Dye Amines for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
145	Cyanine 3 Amine [equivalent to Cy3® Amine]	1 mg	555	565	150,000	0.073
139	Cyanine 3.5 Amine [equivalent to Cy3.5® Amine]	1 mg	581	596	125,000	0.178
155	Cyanine 5 Amine [equivalent to Cy5® Amine]	1 mg	649	665	250,000	0.030
176	Cyanine 5.5 Amine [equivalent to Cy5.5® Amine]	1 mg	678	701	230,000	0.101
165	Cyanine 7 Amine [equivalent to Cy7® Amine]	1 mg	749	776	275,000	0.036
2006	DABCYL C2 Amine	100 mg	428	N/A	20,000	0.516
810	Dansyl Cadaverine [5-Dimethylaminonaphthalene-1-(N-(5-aminopentyl))sulfonamide]	25 mg	333	518	4,000	0.387
2025	DNP Amine	25 mg	350	N/A	18,000	0.181
610	EDANS Acid [5-((2-Aminoethyl)amino)naphthalene-1-sulfonic Acid]	1 g	335	493	5,500	0.107
615	EDANS Sodium Salt [5-((2-Aminoethyl)aminonaphthalene-1-sulfonic Acid, Sodium Salt]	1 g	335	493	5,500	0.107
128	5-FAM Cadaverine	100 mg	494	521	75,000	0.178
127	5(6)-FAM Cadaverine	100 mg	494	519	75,000	0.172
356	5-TAMRA Cadaverine	5 mg	544	575	75,000	0.178
355	5(6)-TAMRA Cadaverine	25 mg	544	575	75,000	0.187
129	5-FITC Cadaverine	100 mg	492	515	75,000	0.254
2239	Tide Fluor™ 1 Amine [TF1 Amine] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2246	Tide Fluor™ 2 Amine [TF2 Amine] *Superior Replacement for Fluorescein*	1 mg	500	527	75,000	0.091
2269	Tide Fluor™ 3 Amine [TF3 Amine] *Superior Replacement for Cy3®*	1 mg	555	584	75,000	0.179
2286	Tide Fluor™ 4 Amine [TF4 amine] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2279	Tide Fluor™ 5WS Amine [TF5WS Amine] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2292	Tide Fluor™ 6WS Amine [TF6WS Amine] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2331	Tide Fluor™ 7WS Amine [TF7WS Amine] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2336	Tide Fluor™ 8WS Amine [TF8WS Amine] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2192	Tide Quencher™ 1 Amine [TQ1 Amine]	5 mg	490	N/A	20,000	0.194
2202	Tide Quencher™ 2 Amine [TQ2 Amine]	5 mg	515	N/A	21,000	0.120
2222	Tide Quencher™ 3 Amine [TQ3 Amine]	5 mg	570	N/A	22,000	0.091
2061	Tide Quencher™ 4WS Amine [TQ4WS Amine]	1 mg	603	N/A	90,000	0.136
2076	Tide Quencher™ 5WS Amine [TQ5WS Amine]	1 mg	661	N/A	130,000	0.082
2091	Tide Quencher™ 6WS Amine [TQ6WS Amine]	1 mg	704	N/A	130,000	0.102
2106	Tide Quencher™ 7WS Amine [TQ7WS Amine]	1 mg	763	N/A	140,000	0.091
482	Texas Red® Cadaverine	5 mg	582	602	95,000	0.360

6 Label Cysteine Residue Using Fluorescent Dye Maleimides

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Because free thiol (SH) groups, also called mercapto groups, are not present as abundantly as amino groups in most peptides, thiol-reactive reagents often provide a means of selectively modifying a peptide at a defined site. Therefore thiol-reactive dyes are often used to prepare fluorescent peptides 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 thio-ether conjugates even under neutral conditions. Maleimides require conjugation conditions less stringent than those of iodoacetamides, and do not react with histidine and methionine under physiological conditions. For example, most maleimide conjugations can be done at room temperature and neutral pH.

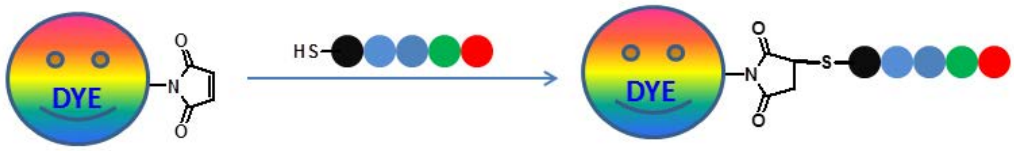

Figure 6.1. The reaction scheme of a peptide with a dye maleimide at cysteine residue.

Table 6.1 Dye Maleimides for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
503	AMCA C2 Maleimide	5 mg	353	455	18,000	0.153
142	Cyanine 3 Maleimide [equivalent to Cy3® Maleimide]	1 mg	555	565	150,000	0.073
149	Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide]	1 mg	581	596	125,000	0.178
152	Cyanine 5 Maleimide [equivalent to Cy5® Maleimide]	1 mg	649	665	250,000	0.030
175	Cyanine 5.5 Maleimide [equivalent to Cy5.5® Maleimide]	1 mg	678	701	230,000	0.101
162	Cyanine 7 Maleimide [equivalent to Cy7® Maleimide]	1 mg	749	776	275,000	0.036
2008	DABCYL C2 Maleimide	25 mg	428	N/A	20,000	0.516
2026	DNP Maleimide	25 mg	350	N/A	18,000	0.181
617	EDANS C2 Maleimide	25 mg	335	493	5,500	0.107
130	Fluorescein-5-maleimide	25 mg	491	514	74,000	0.275
2242	Tide Fluor™ 1 Maleimide [TF1 Maleimide] *Superior Replacement for EDANS*	5 mg	345	442	20,000	0.187
2247	Tide Fluor™ 2 Maleimide [TF2 Maleimide] *Superior Replacement for Fluorescein*	1 mg	500	527	75,000	0.091
2270	Tide Fluor™ 3 Maleimide [TF3 Maleimide] *Superior Replacement for Cy3®*	1 mg	555	584	75,000	0.179
2287	Tide Fluor™ 4 Maleimide [TF4 Maleimide] *Superior Replacement for ROX and Texas Red®*	1 mg	590	618	90,000	0.436
2280	Tide Fluor™ 5WS Maleimide [TF5WS Maleimide] *Superior Replacement for Cy5®*	1 mg	649	664	250,000	0.027
2293	Tide Fluor™ 6WS Maleimide [TF6WS Maleimide] *Superior Replacement for Cy5.5®*	1 mg	676	695	220,000	0.101
2332	Tide Fluor™ 7WS Maleimide [TF7WS Maleimide] *Superior Replacement for Cy7®*	1 mg	749	775	275,000	0.049
2337	Tide Fluor™ 8WS Maleimide [TF8WS Maleimide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2196	Tide Quencher™ 1 Maleimide [TQ1 Maleimide]	5 mg	490	N/A	20,000	0.194
2206	Tide Quencher™ 2 Maleimide [TQ2 Maleimide]	5 mg	515	N/A	21,000	0.120
2226	Tide Quencher™ 3 Maleimide [TQ3 Maleimide]	5 mg	570	N/A	22,000	0.091
2064	Tide Quencher™ 4WS Maleimide [TQ4WS Maleimide]	1 mg	603	N/A	90,000	0.205
2079	Tide Quencher™ 5WS Maleimide [TQ5WS Maleimide]	1 mg	661	N/A	130,000	0.082
2094	Tide Quencher™ 6WS Maleimide [TQ6WS Maleimide]	1 mg	704	N/A	130,000	0.102
2109	Tide Quencher™ 7WS Maleimide [TQ7WS Maleimide]	1 mg	763	N/A	140,000	0.091

7 Direct Labeling of Asp, Glu & Lys Residues Using Dye-Labeled FMOC Amino Acids

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FMOC-Asp(Dye)-OH

Although there are many fluorescent reagents developed for labeling N-terminus of peptides, very few reagents are available for labeling in-sequence amino acids such as aspartic acid, glutamic acid and lysine residues. AAT Bioquest now offers a variety of fluorescent FMOC-Asp(Dye)-OH derivatives and amine-containing labeling reagents to facilitate the in-sequence labeling of aspartic acid residues of peptides.

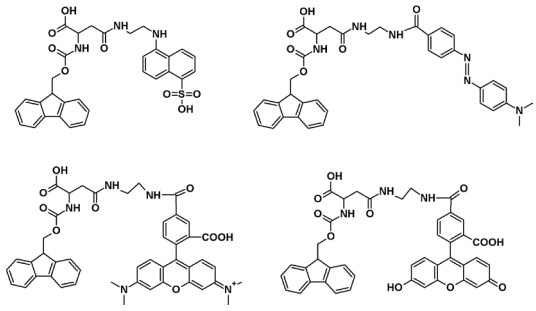

Figure 7.1. The chemical structures of typical FMOC-Asp(Dye)-OH reagents.

FMOC-Glu(Dye)-OH

Besides FMOC-Asp(Dye)-OH peptide-labeling reagents listed on the left column, AAT Bioquest also offers a variety of fluorescent FMOC-Glu(Dye)-OH derivatives and amine-containing labeling reagents to facilitate the in-sequence labeling of glutamic acid residues of peptides. These FMOC-Glu(Dye)-OH reagents can be used as FMOC-Asp(Dye)-OH building blocks.

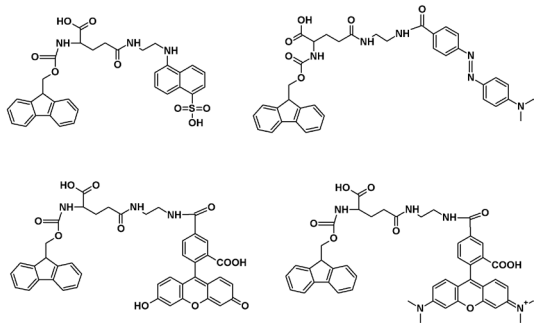

Figure 7.2. The chemical structures of typical FMOC-Glu(Dye)-OH reagents.

Table 7.1 FMOC-Asp(Dye)-OH & FMOC-Glu(Dye)-OH Derivatives for Labeling Aspartic Acid and Glutamic Acid Residues

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
5204	FMOC-Asp(DABCYL)-OH	100 mg	428	N/A	20,000	0.516
5000	FMOC-Asp(EDANS)-OH	100 mg	335	493	5,500	0.107
5001	FMOC-Asp(EDANS)-OH	1 g	335	493	5,500	0.107
5002	FMOC-Asp(EDANS)-OH	5 g	335	493	5,500	0.107
5004	FMOC-Asp(5-FAM)-OH	100 mg	494	521	75,000	0.178
5003	FMOC-Asp(5/6-FAM)-OH	100 mg	494	519	75,000	0.172
5006	FMOC-Asp(5-TAMRA)-OH	100 mg	544	572	75,000	0.178
5005	FMOC-Asp(5/6-TAMRA)-OH	100 mg	544	572	75,000	0.187
5007	FMOC-Asp(TF3)-OH	100 mg	554	583	75,000	0.179
5206	FMOC-Asp(TQ2)-OH	100 mg	515	N/A	21,000	0.120
5208	FMOC-Asp(TQ3)-OH	100 mg	570	N/A	22,000	0.091
5234	FMOC-Glu(DABCYL)-OH	100 mg	428	N/A	20,000	0.516
5009	FMOC-Glu(EDANS)-OH	100 mg	335	493	5,500	0.107
5010	FMOC-Glu(EDANS)-OH	1 g	335	493	5,500	0.107
5011	FMOC-Glu(EDANS)-OH	5 g	335	493	5,500	0.107
5013	FMOC-Glu(5-FAM)-OH	100 mg	494	521	75,000	0.178
5015	FMOC-Glu(5-TAMRA)-OH	100 mg	544	572	75,000	0.178
5012	FMOC-Glu(5/6-FAM)-OH	100 mg	494	519	75,000	0.172
5014	FMOC-Glu(5/6-TAMRA)-OH	100 mg	544	572	75,000	0.187
5016	FMOC-Glu(TF3)-OH	100 mg	554	583	75,000	0.179
5236	FMOC-Glu(TQ2)-OH	100 mg	515	N/A	21,000	0.120
5238	FMOC-Glu(TQ3)-OH	100 mg	570	N/A	22,000	0.091

FMOC-Lys(Dye)-OH

Although there are many fluorescent reagents developed for labeling N-terminus of peptides, very few reagents are available for labeling in-sequence amino acids such as aspartic acid, glutamic acid and lysine residues. AAT Bioquest now offers a variety of fluorescent FMOC-Lys(Dye)-OH derivatives to facilitate the in-sequence labeling of lysine residues of peptides. In addition, all our amine-reactive fluorescent dyes can also be used to label in-sequence lysine residues if the N-terminal amino group is blocked.

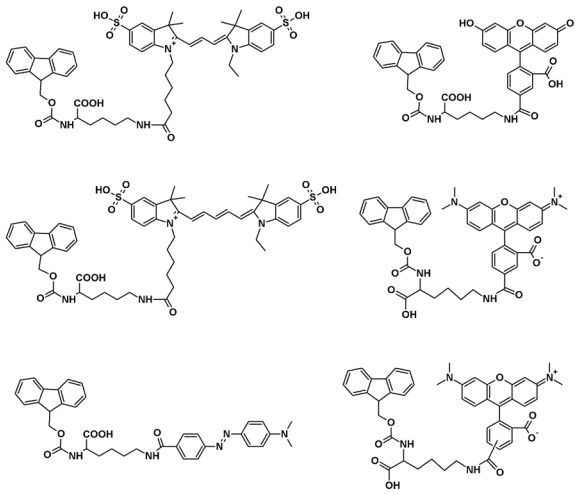

Figure 7.3. The chemical structures of typical FMOC-Lys(Dye)-OH reagents.

An example of using FMOC-Lys(Dye)-OH reagent [doi:10.1021/bc400011v]: β-Ala-Lys(5-FAM)-dPEG6-Fmoc was synthesized on FMOC-β-Ala-Wang resin (loading capacity, 0.36 mmol/g). FMOC-N-amido-dPEG6 acid (MW = 575.65 Da, FMOC-dPEG6-OH) was purchased from Quanta Biodesign Ltd. (Powell, OH). The Wang resin (0.1 g) was swollen with DMF in PD-10 column for 1 hour, prior to coupling. The N-terminal FMOC group was removed using piperidine/DMF (1:4 v/v, 2 × 4 mL, 10 minutes each), followed by washing with DMF. FMOC-Lys(5-FAM)-OH (7.3 mg, 0.01 mmol) was activated with PyBOP (20.8 mg, 0.04 mmol) and HOBt (5.4 mg, 0.04 mmol) in DMF, mixed with DIPEA (13.2 µL, 0.08 mmol), and transferred to a PD-10 column for coupling. The column was placed on a shaker for 4 hours. Next, the resin was washed with DMF and treated with acetic anhydride (24.5 µL, 0.26 mmol) and DIPEA (17.2 µL, 0.10 mmol) in DMF for 0.5 hour. Prior to dPEG coupling, the terminal FMOC group was removed. After washing with DMF, FMOC-dPEG6-OH (23.0 mg, 0.04 mmol), PyBOP (20.8 mg, 0.04 mmol), HOBt (5.4 mg, 0.04 mmol), DIPEA (13.2 µL, 0.08 mmol) in DMF were transferred to a PD-10 column to couple dPEG. The Kaiser test was used to monitor completion of the coupling steps. Finally, the resin was washed with methanol and DCM and then dried in vacuum. The peptide was purified on an RP-HPLC system using a Symmetry C18 column (4.6 mm × 150 mm, 100 Å, particle size 5 µm). The following mobile phase was used: solvent A, 0.05% aqueous TFA; solvent B, acetonitrile containing 0.05% TFA. A linear gradient of 5–30% of B was applied for 60 minutes at a flow rate of 1 mL/min. The UV absorptions were monitored at 220 and 250 nm. MALDI-TOF-MS calculated [M + H+]: 1133.45. Found: 1132.98.

Table 7.2 FMOC-Lys(Dye)-OH Derivatives for Labeling Lysine Residues

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
5054	FMOC-Lys(Cy3)-OH	100 mg	554	578	150,000	0.073
5055	FMOC-Lys(Cy5)-OH	100 mg	649	664	250,000	0.030
5040	FMOC-Lys(DABCYL)-OH	100 mg	428	N/A	20,000	0.516
5041	FMOC-Lys(DABCYL)-OH	1 g	428	N/A	20,000	0.516
5043	FMOC-Lys(5-FAM)-OH	100 mg	494	521	75,000	0.178
5045	FMOC-Lys(5-TAMRA)-OH	100 mg	544	572	75,000	0.178
5042	FMOC-Lys(5/6-FAM)-OH	1 g	494	519	75,000	0.172
5044	FMOC-Lys(5/6-TAMRA)-OH	100 mg	544	572	75,000	0.187
5050	FMOC-Lys(TF2-Boc)-OH	100 mg	501	526	75,000	0.091
5051	FMOC-Lys(TF3)-OH	100 mg	554	583	75,000	0.179
5070	FMOC-Lys(5-FITC)-OH	100 mg	492	515	75,000	0.254
5256	FMOC-Lys(TQ2)-OH	100 mg	515	N/A	21,000	0.120
5258	FMOC-Lys(TQ3)-OH	100 mg	570	N/A	22,000	0.091

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Clickable Dyes for Labeling Peptides

8

"Click Chemistry" is a term that was introduced by K. B. Sharpless in 2001 to describe reactions that have high 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.

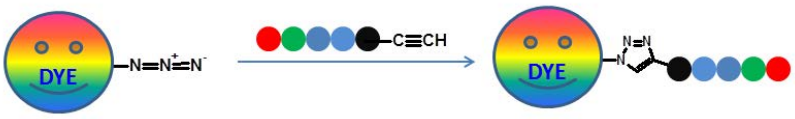

Figure 8.1 The reaction scheme of a peptide alkyne with a dye azide.

Table 8.1 Dye Azides for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
508	AMCA Azide	1 mg	353	455	18,000	0.153
143	Cyanine 3 Azide [equivalent to Cy3® Azide]	1 mg	555	565	150,000	0.073
153	Cyanine 5 Azide [equivalent to Cy5® Azide]	1 mg	649	665	250,000	0.030
178	Cyanine 5.5 Azide [equivalent to Cy5.5® Azide]	1 mg	678	701	230,000	0.101
163	Cyanine 7 Azide [equivalent to Cy7® Azide]	1 mg	749	776	275,000	0.036
131	5-FAM Azide	10 mg	494	521	75,000	0.178
1091	iFluor™ 647 Azide	1 mg	649	665	250,000	0.036
486	5-TAMRA Azide	5 mg	547	573	75,000	0.178
484	Texas Red® Azide *Single Isomer*	5 mg	588	601	95,000	0.360
2236	Tide Fluor™ 1 Azide [TF1 Azide]	5 mg	345	442	20,000	0.187
2252	Tide Fluor™ 2 Azide [TF2 Azide]	1 mg	500	527	75,000	0.091
2254	Tide Fluor™ 3 Azide [TF3 Azide]	1 mg	555	584	75,000	0.178
2300	Tide Fluor™ 4 Azide [TF4 Azide]	1 mg	590	618	90,000	0.436
2275	Tide Fluor™ 5WS Azide [TF5WS Azide]	1 mg	649	664	250,000	0.027
2302	Tide Fluor™ 6WS Azide [TF6WS Azide]	1 mg	676	695	220,000	0.101
2304	Tide Fluor™ 7WS Azide [TF7WS Azide]	1 mg	749	775	275,000	0.049
2306	Tide Fluor™ 8WS Azide [TF8WS azide] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2188	Tide Quencher™ 1 Azide [TQ1 Azide]	5 mg	515	N/A	20,000	0.194
2211	Tide Quencher™ 2 Azide [TQ2 Azide]	5 mg	515	N/A	21,000	0.120
2231	Tide Quencher™ 3 Azide [TQ3 Azide]	5 mg	570	N/A	22,000	0.091
2068	Tide Quencher™ 4WS Azide [TQ4WS Azide]	1 mg	603	N/A	90,000	0.136
2082	Tide Quencher™ 5WS Azide [TQ5WS Azide]	1 mg	661	N/A	130,000	0.082
2097	Tide Quencher™ 6WS Azide [TQ6WS Azide]	1 mg	704	N/A	130,000	0.102
2112	Tide Quencher™ 7WS Azide [TQ7WS Azide]	1 mg	763	N/A	140,000	0.091

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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 a peptide, 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 an alkyne derivative, but functional moiety can serve as either 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 a peptide 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 peptides. These clickable reagents include both common fluorescent dyes (e.g., fluoresceins, rhodamines and cyanines) and non-fluorescent quenchers. Our Tide Fluor™ and Tide Quencher™ dyes are specifically optimized for preparing novel FRET substrates.

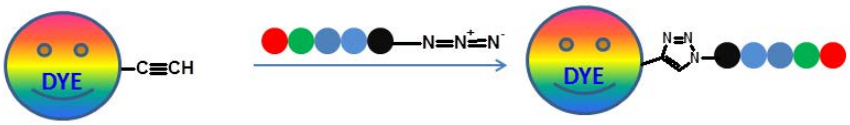

Figure 8.2 The reaction scheme of a peptide azide with a dye alkyne.

Table 8.2 Dye Alkynes for Labeling Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @280 nm
507	AMCA Alkyne	1 mg	353	455	18,000	0.153
144	Cyanine 3 Alkyne [equivalent to Cy3® Alkyne]	1 mg	555	565	150,000	0.073
154	Cyanine 5 Alkyne [equivalent to Cy5® Alkyne]	1 mg	649	665	250,000	0.030
179	Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne]	1 mg	678	701	230,000	0.101
164	Cyanine 7 Alkyne [equivalent to Cy7® Alkyne]	1 mg	749	776	275,000	0.036
132	5-FAM Alkyne	10 mg	494	521	75,000	0.178
487	5-TAMRA Alkyne	5 mg	547	573	75,000	0.178
485	Texas Red® Alkyne *Single Isomer*	5 mg	588	601	95,000	0.360
2237	Tide Fluor™ 1 Alkyne [TF1 Alkyne]	5 mg	345	442	20,000	0.187
2253	Tide Fluor™ 2 Alkyne [TF2 Alkyne]	1 mg	500	527	75,000	0.091
2255	Tide Fluor™ 3 Alkyne [TF3 Alkyne]	1 mg	555	584	75,000	0.178
2301	Tide Fluor™ 4 Alkyne [TF4 Alkyne]	1 mg	590	618	90,000	0.436
2276	Tide Fluor™ 5WS Alkyne [TF5WS Alkyne]	1 mg	649	664	250,000	0.027
2303	Tide Fluor™ 6WS Alkyne [TF6WS Alkyne]	1 mg	676	695	220,000	0.101
2305	Tide Fluor™ 7WS Alkyne [TF7WS Alkyne]	1 mg	749	775	275,000	0.049
2307	Tide Fluor™ 8WS Alkyne [TF8WS Alkyne] *Near Infrared Emission*	1 mg	775	807	250,000	0.109
2189	Tide Quencher™ 1 Alkyne [TQ1 Alkyne]	5 mg	515	N/A	20,000	0.194
2212	Tide Quencher™ 2 Alkyne [TQ2 Alkyne]	5 mg	515	N/A	21,000	0.120
2232	Tide Quencher™ 3 Alkyne [TQ3 Alkyne]	5 mg	570	N/A	22,000	0.091
2069	Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne]	1 mg	603	N/A	90,000	0.130
2083	Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne]	1 mg	661	N/A	130,000	0.082
2098	Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne]	1 mg	704	N/A	130,000	0.102
2113	Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne]	1 mg	763	N/A	140,000	0.091

9 Dye Selection Guides for Labeling Peptides

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Dye Selection Guide for Preparing Fluorescent Peptides with Desired Excitation and Emission Properties*

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

Dye Selection Guide for Preparing FRET Peptides

Acceptors / Donors	TQ1 BHQ®-0 QSY®35 DABCYL	TQ2 BHQ®-1	TQ3 BHQ®-2 QSY®7 QSY®9	TQ4 BHQ®-3 QSY®21	TQ5	TQ6	TQ7
Tide Fluor™ 1 (TF1) **iFluor™ 350** Alexa Fluor® 350 DyLight™ Fluor 350 EDANS MCA	☺						
Tide Fluor™ 2 (TF2) **iFluor™ 488** Alexa Fluor® 488 ATTO 488 DyLight™ Fluor 488 FAM/TITC Cy2®		☺					
Helix Fluor™ 555 **iFluor™ 532** Hex TET JOE VIC			☺				
Tide Fluor™ 3 (TF3) **iFluor™ 555** **Helix Fluor™ 575** Alexa Fluor® 546 & 555 ATTO 550 & 565 DyLight™ Fluor 550 Cy3®/NED/TAMRA			☺				
Tide Fluor™ 4 (TF4) **iFluor™ 594** **California Red™** **SunRed™** Alexa Fluor® 594 DyLight™ Fluor 594 ROX Texas Red® Texas Red®-X				☺			
Tide Fluor™ 5 (TF5) **iFluor™ 647** Alexa Fluor® 647 DyLight™ Fluor 650 Cy5®					☺		
Tide Fluor™ 6 (TF6) **iFluor™ 680** IRDye® 700 Alexa Fluor® 680 DyLight™ Fluor 680 Cy5.5®						☺	
Tide Fluor™ 7 (TF7) **iFluor™ 750** Alexa Fluor® 750 DyLight™ Fluor 755 IRDye® 800 Cy7®							☺

Best to use OK to use Not recommended

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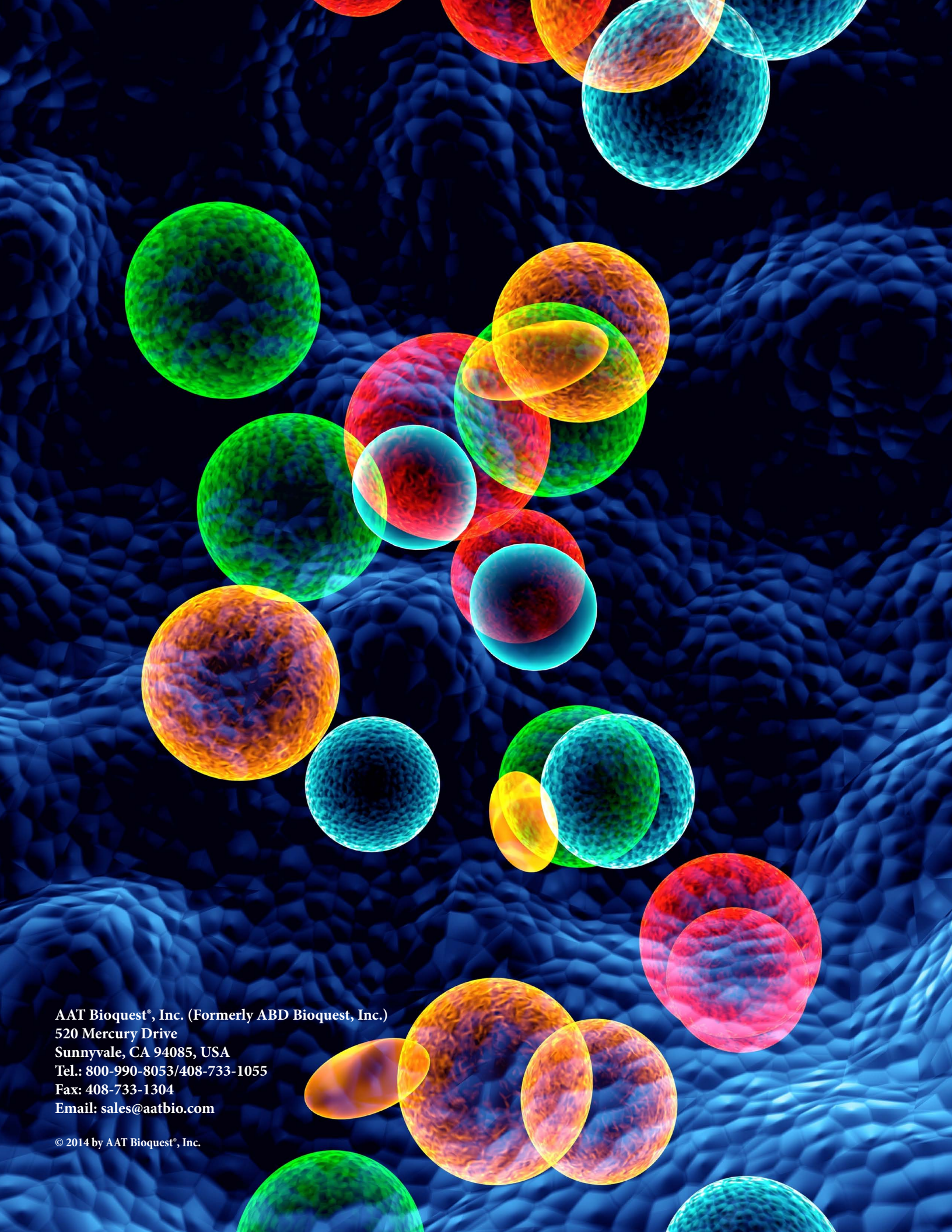

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