2014 • Volume 3 • Issue 1

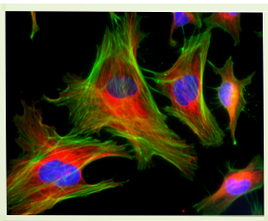
AssayWise Letters

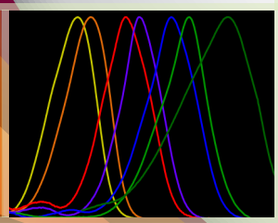
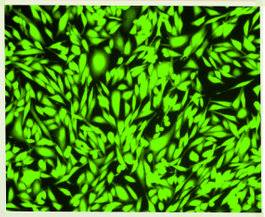
New Product Highlights
Total Solution for NAD/NADH & NADP/NADPH Detection
Sphingomyelinase Assays

Optimized FRET Building Blocks
Tide Fluor™ Dyes, Optimized FRET Donors
Tide Quencher™ Dyes, Optimized FRET Acceptors

Cell-Based Assays
Cal-520™, the Best Green Fluorescent Ca^{2+} Dye

Labeling Probes
California Red™ & SunRed™, Superior Replacements for Texas Red®
Click Chemistry Building Blocks



AAT Bioquest®
Advancing Assay & Test Technologies

Table of Contents

New Product Highlights

Optimized FRET Building Blocks

Cell-Based Assays

Labeling Probes

From the President of AAT Bioquest

AAT Bioquest, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. AAT Bioquest offers a rapidly expanding list of enabling products. ***AssayWise Letters*** is a platform for AAT Bioquest to introduce its newest products and services, and to update the new applications of our existing products. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest also offers custom service to meet the distinct needs of each customer.

It is my greatest pleasure to welcome you to this new issue of our ***AssayWise Letters***. While we continue to rapidly expand, our core value remains the same: **Innovation and Customer Satisfaction**. We are committed to being the provider of novel biological detection solutions. We promise you 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 feedback and suggestions from you.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

Total Solution for NAD/NADH & NADP/NADPH Detection

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+ and NAD+ is the oxidized form of NADH. NAD forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are based on monitoring absorption changes in NADH or NADPH at 340 nm. The short UV wavelengths of NAD/NADH and NADP/NADPH assays make the traditional methods suffer low sensitivity and high interference.

AAT Bioquest offers the most comprehensive product portfolio for NAD/NADH and NADP/NADPH detection as summarized in Table 1.1. All our NAD/NADH and NADP/NADPH assay kits are in a mix and read format with minimal hands-on time required. These kits have either significantly improved sensitivity and dynamic range or less interference from biological samples compared to the commercial assay kits from other vendors. Through our continuous improvement and innovation, our new NAD/NADH and NADP/NADPH assay kit 15275 and kit 15276 achieve outstanding sensitivity.

Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15275) provides a convenient method for detecting total NAD and NADH. There is no need to purify NAD and NADH from sample mix. The enzymes in the system specifically recognize NAD and NADH in an enzyme cycling reaction, and thus significantly increase detection sensitivity. The NAD/NADH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NAD/NADH reduction. The maximum absorbance increases to ~635 nm when enhancer is added to the assay system. The absorption of the NAD/NADH probe is directly proportional to the concentration of NAD/NADH. Amplite™ Colorimetric Total NAD and NADH Assay Kit detects as little as 0.1 µM total NAD and NADH in a 100 µL assay volume. Compared to Amplite™ Colorimetric Total NAD and NADH Assay Kit 15258, Kit 15275 demonstrates higher sensitivity.

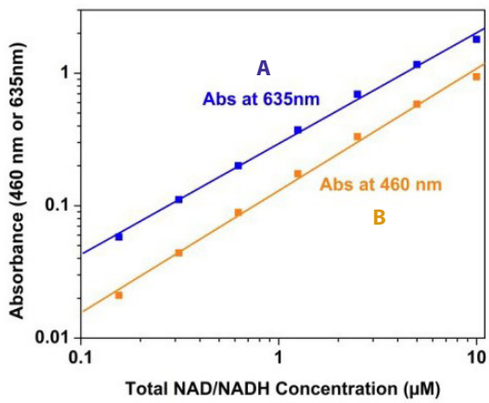


Figure 1.1. Total NAD and NADH dose responses were measured with Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15275) in a 96-well white/clear bottom plate. As low as 0.1 µM total NAD and NADH was detected with 1 hour incubation (n=3). A: Absorbance measured at 460 nm, B: Absorbance measured at 635 nm after adding enhancer to each well.

Table 1.1. NAD/NADH & NADP/NADPH Assay Comparison

Cat. #	Product Name	Assay Target	Detection Mode	Detection Limit	Dynamic Range
15280	Amplite™ Fluorimetric NAD Assay Kit *Blue Fluorescence*	NAD	Fluorescence	0.03 µM	0.03-10 µM
15271	Amplite™ Colorimetric NADH Assay Kit	NADH	Absorption	3 µM	1-200 µM
15261	Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence*	NADH	Fluorescence	1 µM	0-100 µM
15258	Amplite™ Colorimetric Total NAD and NADH Assay Kit	NAD+NADH	Absorption	0.3 µM	0-10 µM
15275	Amplite™ Colorimetric Total NAD and NADH Assay Kit *Enhanced Sensitivity*	NAD+NADH	Absorption	0.1 µM	0.1-10 µM
15257	Amplite™ Fluorimetric Total NAD and NADH Assay Kit *Red Fluorescence*	NAD+NADH	Fluorescence	0.1 µM	0-3 µM
15273	Amplite™ Colorimetric NAD/NADH Ratio Assay Kit	NAD/NADH Ratio	Absorption	0.1 µM	0.1-10 µM
15263	Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit	NAD/NADH Ratio	Fluorescence	0.1 µM	0-3 µM
15281	Amplite™ Fluorimetric NADP Assay Kit *Blue Fluorescence*	NADP	Fluorescence	0.03 µM	0.03-10 µM
15272	Amplite™ Colorimetric NADPH Assay Kit	NADPH	Absorption	3 µM	1-200 µM
15262	Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence*	NADPH	Fluorescence	1 µM	0-100 µM
15260	Amplite™ Colorimetric Total NADP and NADPH Assay Kit	NADP+NADPH	Absorption	0.1 µM	0-3 µM
15276	Amplite™ Colorimetric Total NADP and NADPH Assay Kit *Enhanced Sensitivity*	NADP+NADPH	Absorption	0.03 µM	0.03-1 µM
15259	Amplite™ Fluorimetric Total NADP and NADPH Assay Kit *Red Fluorescence*	NADP+NADPH	Fluorescence	0.01 µM	0-3 µM
15274	Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit	NADP/NADPH Ratio	Absorption	0.03 µM	0.03-1 µM
15264	Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit *Red Fluorescence*	NADP/NADPH Ratio	Fluorescence	0.01 µM	0-3 µM

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Amplite™ Colorimetric Total NADP/NADPH Assay Kit (Cat# 15276) provides a convenient method for sensitive detection of NADP, NADPH and their ratio. Compared to Amplite™ Colorimetric Total NADP/NADPH Assay Kit 15260, Kit 15276 demonstrates higher sensitivity.

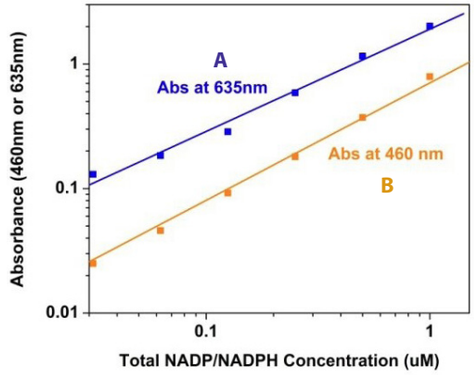


Figure 1.2. Total NADP and NADPH dose responses were measured with Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat# 15276) in a 96-well white/clear bottom plate. As low as 0.03 µM total NADP/NADPH was detected with 1 hour incubation (n=3). A: Absorbance measured at 460 nm; B: Absorbance measured at 635 nm after adding enhancer to each well.

AAT Bioquest has recently developed a set of fluorogenic probes that have excellent responses to NAD and NADP respectively. The new probes enabled us to introduce the two newest NAD and NADP assay kits (Cat# 15280 and Cat# 15281). These two assay kits can be readily used for the high throughput screening of enzymes that use either NAD or NADP as a cofactor. They may enable the NAD and NADP detection in live cells.

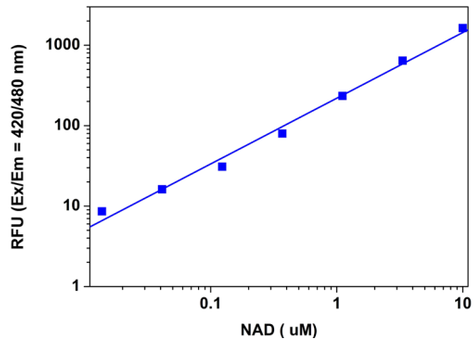


Figure 1.3. NAD dose responses were measured with Amplite™ Fluorimetric NAD Assay Kit (Cat# 15280) in a 96-well black/solid bottom plate. As low as 30 nM NAD was detected with 20 minute incubation (n=3).

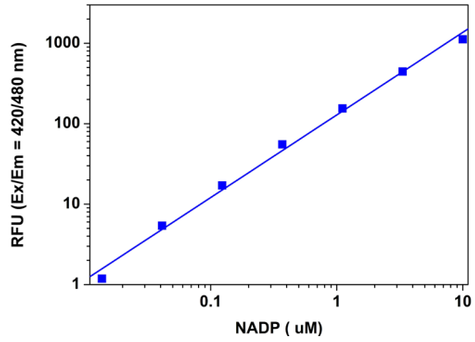


Figure 1.4. NADP dose responses were measured with Amplite™ Fluorimetric NADP Assay Kit (Cat# 15281) in a 96-well black/solid bottom plate. As low as 30 nM NADP was detected with 20 minute incubation (n=3).

Table 1.2. NAD/NADH & NADP/NADPH Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
15258	Amplite™ Colorimetric Total NAD and NADH Assay Kit	400 tests	575	N/A
15275	Amplite™ Colorimetric Total NAD and NADH Assay Kit *Enhanced Sensitivity*	400 tests	635	N/A
15260	Amplite™ Colorimetric Total NADP and NADPH Assay Kit	400 tests	575	N/A
15276	Amplite™ Colorimetric Total NADP and NADPH Assay Kit *Enhanced Sensitivity*	400 tests	635	N/A
15273	Amplite™ Colorimetric NAD/NADH Ratio Assay Kit	250 tests	635	N/A
15271	Amplite™ Colorimetric NADH Assay Kit	400 tests	635	N/A
15274	Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit	250 tests	635	N/A
15272	Amplite™ Colorimetric NADPH Assay Kit	400 tests	635	N/A
15257	Amplite™ Fluorimetric Total NAD and NADH Assay Kit *Red Fluorescence*	400 tests	571	585
15259	Amplite™ Fluorimetric Total NADP and NADPH Assay Kit *Red Fluorescence*	400 tests	571	585
15280	Amplite™ Fluorimetric NAD Assay Kit *Blue Fluorescence*	200 tests	422	466
15263	Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit *Red Fluorescence*	250 tests	571	585
15261	Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence*	400 tests	571	585
15281	Amplite™ Fluorimetric NADP Assay Kit *Blue Fluorescence*	200 tests	422	466
15264	Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit *Red Fluorescence*	250 tests	571	585
15262	Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence*	400 tests	571	585
15266	ReadiUse™ NADP Regenerating Kit	1 kit	N/A	N/A
15265	ReadiUse™ NADPH Regenerating Kit	1 kit	N/A	N/A

Sphingomyelinase Assays

Sphingomyelinase (SMase) is an enzyme that is responsible for cleaving sphingomyelin (SM) to phosphocholine and ceramide. Activation of SMases in cells plays an important role in the cellular responses. Five types of sphingomyelinase have been identified based on their cation dependence and pH optima of action. They are lysosomal acid SMase, secreted zinc-dependent acid SMase, magnesium-dependent neutral SMase, magnesium-independent neutral SMase, and alkaline SMase. Among the five types, the lysosomal acidic SMase and the magnesium-dependent neutral SMase are considered major candidates for the production of ceramide in the cellular response to stress.

Amplite™ Colorimetric Sphingomyelinase Assay Kit (Cat# 13620) provides a sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses Amplite™ Blue as a colorimetric probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin by sphingomyelinase (SMase). It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The absorbance of Amplite™ Blue at 655 nm is proportional to the formation of phosphocholine, therefore to the SMase activity. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

Amplite™ Fluorimetric Sphingomyelinase Assay Kit (Cat# 13621) provides the most sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin by sphingomyelinase (SMase). It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the SMase activity. Amplite™ Red enables the assay readable either in fluorescence intensity mode or in absorbance mode. The kit is an optimized "mix and read" assay that can be used for real time monitoring of SMase activities.

Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit (Cat# 13622) provides one of the most sensitive methods for detecting acidic SMase activity or screening its inhibitors. The kit uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the SMase activity. It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The kit is an optimized "mix and read" assay which is compatible with HTS liquid handling instruments.

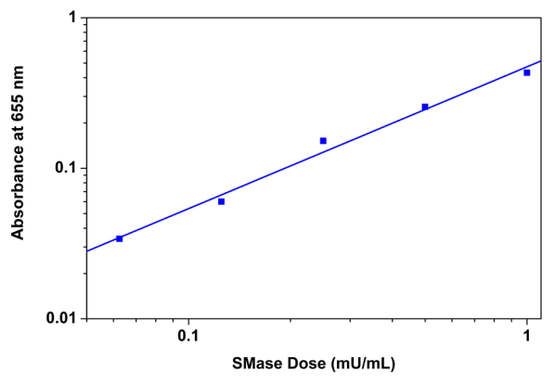


Figure 1.5. Sphingomyelinase dose responses were measured in a 96-well white wall/clear bottom plate with Amplite™ Colorimetric Sphingomyelinase Assay Kit (Cat# 13620) using a SpectraMax® microplate reader (Molecular Devices). As low as 0.08 mU/mL sphingomyelinase was detected with 60 minutes incubation (n=3).

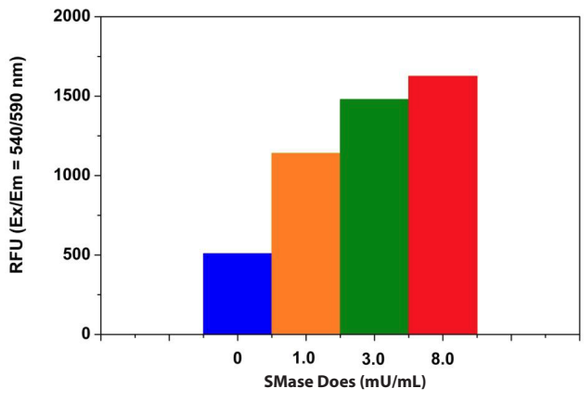


Figure 1.6. Sphingomyelinase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Sphingomyelinase Assay Kit (Cat# 13621) using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.15 mU/mL sphingomyelinase was detected with 60 minutes incubation (n=3).

Table 1.3 Sphingomyelinase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13620	Amplite™ Colorimetric Sphingomyelinase Assay Kit *Blue Color*	200 tests	655	N/A
13622	Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585
13625	Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*	100 tests	571	585
13621	Amplite™ Fluorimetric Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Tide Fluor™ Dyes, Optimized FRET Donors

Although EDANS, FAM, TAMRA, ROX, Cy3® and Cy5® have been widely used to develop a variety of FRET peptide and FRET oligonucleotide probes, there are still some limitations in the use of these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS have severely limited its sensitivity for developing protease assays and nucleic acid detection probes. Compared to EDANS, fluorescein-based probes (such as FAM, HEX, JOE and TET) have stronger absorption and fluorescence. However the fluorescence of fluorescein-based probes is strongly dependent on pH. They only exhibit the strongest fluorescence at higher pH. This pH dependence makes the fluorescein-based fluorescent probes inconvenient for the assays that require low pH. In addition, most of fluorescein-based probes have quite low photostability, which limits their applications in fluorescence imaging.

Among cyanine dyes, non-sulfonated Cy3® and Cy5® are widely used for developing a variety of peptide and oligonucleotide probes, but they have quite low fluorescence quantum yields in aqueous media. The sulfonated Cy3® and Cy5® have improved fluorescence quantum yields than those of non-sulfonate cyanines. Alexa Fluor™ dyes have improved performance, but are extremely expensive, thus are unpractical for preparing peptide and oligonucleotide conjugates in some cases.

To address these limitations, AAT Bioquest has developed Tide Fluor™ donor dyes that are optimized as building blocks for developing FRET peptides and FRET oligonucleotides for a variety of biological applications. Our Tide Fluor™ dyes (such as TF1, TF2, TF3, TF4, TF5, TF6, TF7 and TF8) have strong fluorescence and good photostability. TF2 dyes have the similar excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily used for the biological applications done with fluoresceins. TF2 dyes have much stronger fluorescence at physiological conditions and they are much more photostable than FAM probes. 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. Tide Fluor™ dyes have almost identical spectral properties to Alexa Fluor® dyes as discussed below. However, on oligonucleotides and peptides, TF3 dyes are much brighter and more photostable than Cy3®, Alexa Fluor® 555 and DyLight™ 555. We recommend you try our Tide Fluor™ dyes that are optimized for labeling oligonucleotides and peptides at much lower cost with comparable performance to Alexa Fluor® dyes.

Key Features of Tide Fluor™ Dyes

- *Optimized to pair with Tide Quencher™ dark acceptors*
- *Stronger fluorescence intensity to enhance assay sensitivity*
- *pH-insensitive and environment-insensitive fluorescence*
- *Higher photostability to improve fluorescence imaging*
- *Adjustable water solubility*
- *A variety of reactive forms available for conjugations*

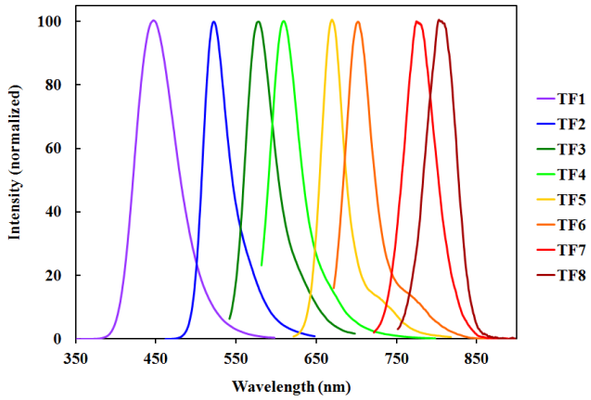


Figure 2.1. The normalized fluorescence spectra of Tide Fluor™ dyes.

Table 2.1 Tide Fluor™ Dye Equivalents of Common Dyes

If you are using	Try this Tide Fluor™ dye
Alexa Fluor® 350, AMCA, DyLight™ 350	Tide Fluor™ 1 [TF 1]
Alexa Fluor® 488, Cy2®, FITC, DyLight™ 488	Tide Fluor™ 2 [TF 2]
Alexa Fluor® 555, Cy3®, DyLight™ 550, TRITC	Tide Fluor™ 3 [TF 3]
Alexa Fluor® 594, DyLight™ 594, Texas Red®	Tide Fluor™ 4 [TF 4]
Alxea Fluor® 647, Cy5®, DyLight™ 650	Tide Fluor™ 5 [TF 5]
Alexa Fluor® 680, Cy5.5®, IRDye® 700, DyLight™ 680	Tide Fluor™ 6 [TF 6]
Alexa Fluor® 750, Cy7®, DyLight™ 750	Tide Fluor™ 7 [TF 7]
Alexa Fluor® 790, DyLight™ 800, IRDye® 800	Tide Fluor™ 8 [TF 8]

Table 2.2 Tide Fluor™ Dyes for Developing FRET Probes

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# 2249 (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)

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Tide Quencher™ Dyes, Optimized FRET Acceptors

Although DABCYL has been used to develop a variety of FRET applications, its low quenching efficiency of longer wavelength dyes (such as fluoresceins, rhodamines and cyanines) has limited its use in the development of sensitive fluorogenic FRET probes. Additionally, the absorption spectrum of DABCYL is environment-sensitive. AAT Bioquest has developed the robust Tide Quencher™ acceptor dyes for the development of longer wavelength FRET probes. These Tide Quencher™ dark FRET acceptors (such as TQ1, TQ2, TQ3, TQ4, TQ5, TQ6 and TQ7) are optimized to pair with our Tide Fluor™ dyes and the classic fluorophores (such as AMCA, EDANS, FAM, TAMRA, HEX, JOE, TET, ROX, Cy3®, Cy5® and Cy7®). Like our Tide Fluor™ donor dyes, our Tide Quencher™ acceptor dyes are much more cost-effective with comparable or even better performance for your desired biological applications than other similar products on the market.

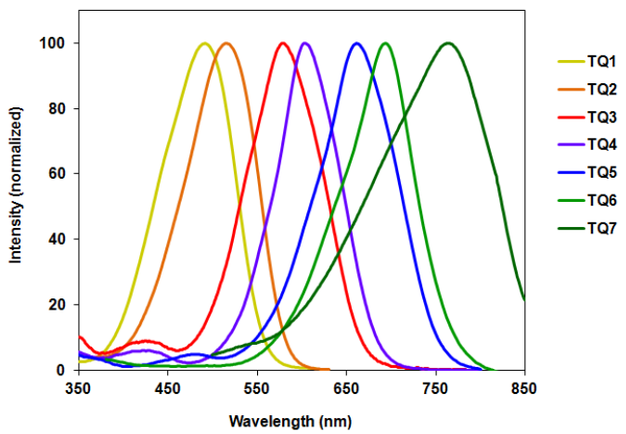


Figure 2.2. The normalized absorption spectra of TQ1, 2, 3, 4, 5, 6 and 7**.**

Besides the broad applications in the development of Molecular Beacon probes, our Tide Quencher™ dyes have also been used to develop various protease substrates such as HIV protease, MMPs and secretases. In some cases, they have demonstrated greatly improved enzyme performance. This may be partly due to the red-shifted absorption spectrum that overlaps better with the emission spectra of fluoresceins, rhodamines and cyanines. Tide Quencher™ dyes are a great choice for you to eliminate the limitations of classic quenchers. As excellent dark quenchers, Tide Quencher™ dyes are individually optimized to pair with all the popular fluorescent dyes such as fluoresceins, rhodamines and cyanines. Our Tide Quencher™ series of nonfluorescent dyes cover the full visible spectrum with unusually high efficiency. Among them, 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®.

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.*

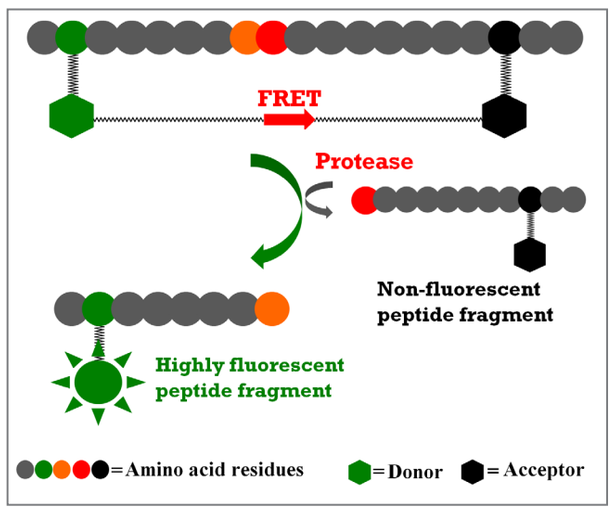


Figure 2.3. The internally quenched FRET peptide substrate is digested by a protease to generate the highly fluorescent peptide fragment. The fluorescence increase is proportional to the protease activity.

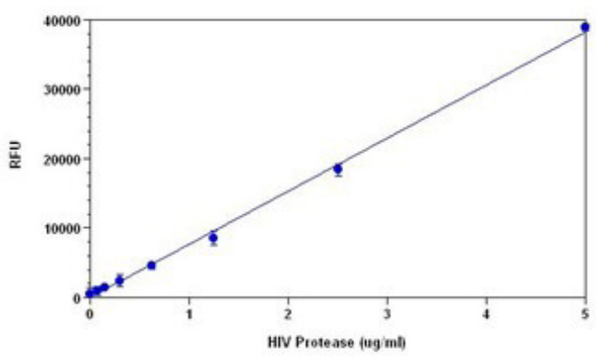


Figure 2.4. HIV protease cleavage of Arg-Glu(5-FAM)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg. The substrate was incubated with HIV protease . Upon HIV protease cleavage, the fluorescence of 5-FAM was recovered and monitored at Ex/Em = 490 /520 nm.

Table 2.3 Tide Quencher™ Dyes for Developing FRET Probes

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)

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Table 2.4 Dye Selection Guide for Preparing FRET Oligonucleotides and Peptides

Donors \ Acceptors	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™ 350 EDANS MCA	☺						
Tide Fluor™ 2 (TF2) **iFluor™ 488** Alexa Fluor® 488 ATTO 488 DyLight™ 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™ 550 Cy3®/NED/TAMRA			☺				
Tide Fluor™ 4 (TF4) **iFluor™ 594** **California Red™** **SunRed™** Alexa Fluor® 594 DyLight™ 594 ROX Texas Red® Texas Red®-X				☺			
Tide Fluor™ 5 (TF5) **iFluor™ 647** Alexa Fluor® 647 DyLight™ 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™ 755 IRDye® 800 Cy7®							☺

California Red™, SunRed™, Tide Fluor™ (TF) and Tide Quencher™ (TQ) are the trademarks of AAT Bioquest. Alexa Fluor®, QSY® and Texas Red® are the trademarks of Invitrogen. Cy2®, Cy3®, Cy5®, Cy5.5® and Cy7® are the trademarks of GE Healthcare. Black Hole Quencher® (BHQ), DyLight™ and IRDye® are the trademarks of ThermoFisher, Bioserach and LI-COR respectively.

Best to use OK to use Not recommended

Cal-520™, the Best Green Fluorescent Ca^{2+} Dye

Cal-520™ provides the most robust homogeneous fluorescence-based assay tool for detecting intracellular calcium mobilization. Cal-520™ AM is a new fluorogenic calcium-sensitive dye with a significantly improved signal to background ratio and intracellular retention compared to the existing green calcium indicators (such as Fluo-3 AM and Fluo-4 AM). The higher signal/background ratio and better intracellular retention make the Cal-520™ calcium assay a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

Our preliminary in-house research indicated that Cal-520™ AM can be readily loaded to a guinea pig heart and stays there for a few hours in the absence of probenecid. The calcium signal can be readily monitored with Cal-520™ AM while it is difficult to observe the calcium signal under the same conditions with other green calcium dyes such as Fluo-3 AM and Fluo-4 AM.

Table 3.1 Spectral Comparison of Fluo-3, Fluo-4, Fluo-8® and Cal-520™

Dye	Ex (nm)	Em (nm)	QY*
Cal-520™	492	514	**0.75**
Fluo-3	506	525	0.15
Fluo-4	493	515	0.16
Fluo-8®	490	514	0.16

*QY = Fluorescence Quantum Yield in the presence of 5 mM calcium citrate.

Key Features of Cal-520™ AM

- *Cal-520™ AM is better retained in live cells than Fluo-3 AM and Fluo-4 AM.*
- *Cal-520™ AM has much higher signal/background ratio than Fluo-3 AM and Fluo-4 AM in cells.*
- *Cal-520™ AM has almost identical spectra to those of Fluo-4 AM.*

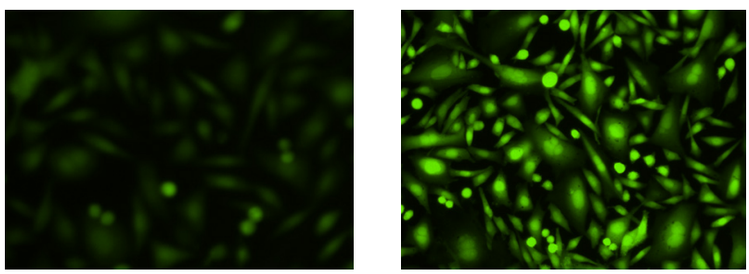


Figure 3.1. Responses of endogenous P2Y receptor to ATP in CHO-M1 cells without probenecid. CHO-M1 cells were seeded overnight at 40,000 cells per 100 µL per well in a Costar 96-well black wall/clear bottom plate. 100 µL of 4 µM Fluo-4 AM (left), Cal 520™ AM (right) in HHBS was added into the wells, and the cells were incubated at 37 °C for 2 hours. The dye loading medium was replaced with 100 µL HHBS and 50 µL of 300 µM ATP were added. The cells were imaged with a fluorescence microscope (Olympus IX71) using FITC channel.

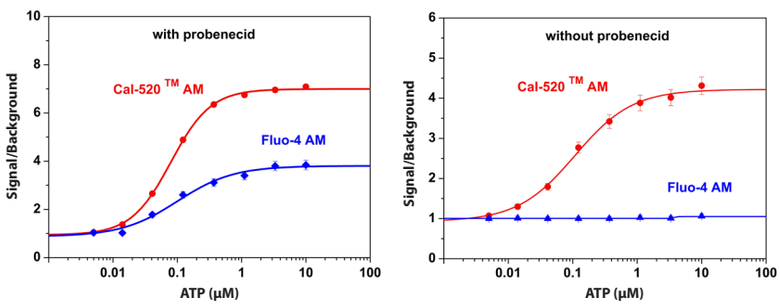


Figure 3.2. ATP-stimulated calcium responses of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-520™ AM (red curve), or Fluo-4 AM (blue curve) respectively with (left) or without probenecid (right) under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100 µL per well in a Costar black wall/clear bottom 96-well plate. 100 µL of 5 µM Fluo-4 AM or Cal 520™ AM in HHBS (with or without 2.5 mm probenecid) was added into the cells, and the cells were incubated at 37 °C for 2 hours. ATP (50 µL/well) was added using FlexStation® to achieve the final indicated concentrations.

Table 3.2. Spectral and Ca^{2+}–Binding Properties of Cal-520™ and Fluo-8® Calcium Indicators

Ca^{2+} Indicator	Excitation	Emission	K_d (Ca^{2+}-Binding)
Cal-520™	492 nm	514 nm	320 nM
Cal-520FF™	492 nm	514 nm	9.8 µM
Fluo-8®	490 nm	514 nm	389 nM
Fluo-8H™	490 nm	514 nm	232 nM
Fluo-8L™	490 nm	514 nm	1.86 µM
Fluo-8FF™	490 nm	514 nm	10 µM

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Recent Publication Highlights of Cal-520™ AM

M. Tada, A. Takeuchi, M. Hashizume, K. Kitamura and M. Kano. **A highly sensitive fluorescent indicator dye for calcium imaging of neural activity *in vitro* and *in vivo*.** *Eur J Neurosci*, Published on January 9, 2014 [DOI: 10.1111/ejn.12476]

Tada et al. used Cal-520 AM calcium imaging to monitor the activities of individual neurons *in vitro* and *in vivo*. Cal-520 AM or Oregon Green 488 BAPTA-1 AM was loaded in neurons under the same conditions. Calcium imaging was performed for more than 30 min after dye injection. Cal-520 AM is sufficiently sensitive to reliably detect single action potentials (APs) both *in vitro* and *in vivo*. In neocortical neurons, Cal-520 calcium signals were linearly correlated with the number of APs, and the SNR was > 6 for *in vitro* slice preparations and > 1.6 for *in vivo* anesthetised mice. In cerebellar Purkinje cells, dendritic calcium transients evoked by climbing fiber inputs were clearly observed in anesthetised mice with a high SNR and fast decay time. Cal-520 AM demonstrated great advantages over Oregon Green BAPTA-1 AM, the most commonly used calcium indicator dye, for monitoring the activity of individual neurons both *in vitro* and *in vivo*.

D. Kodama and A. Togari. **Store-operated calcium entry induced by activation of Gq-coupled alpha1B adrenergic receptor in human osteoblast** *Biochem Biophys Res Comm*. 2013, 437(2), 239–244 [doi:10.1016/j.bbrc.2013.06.047]

Kodama and Togari used Cal-520 AM to study the signal transduction pathway of noradrenaline (NA)-induced $[Ca^{2+}]_i$ elevation in human osteoblast SaM-1 cells. Cal-520 AM was loaded in human osteoblast SaM-1 cells for 30 min with Hanks and 10 mM HEPES buffer (pH 7.4). The fluorescence of Cal-520 was recorded every 2 seconds with 488 nm excitation, and the data were analyzed with ZEN 2009. The authors demonstrated that the intracellular $[Ca^{2+}]_i$ was increased by NA via α1B-AR. The signal pathway of NA-induced $[Ca^{2+}]_i$ elevation was monitored using Ca^{2+} fluorescence imaging in SaM-1 cells. The authors concluded the $[Ca^{2+}]_i$ elevation was mediated by Gq protein-coupled α1B-AR, and Ca^{2+} influx is the predominant pathway of NA-induced $[Ca^{2+}]$i elevation. Ca^{2+} influx through store-operated Ca^{2+} channels plays a critical role in the signal transduction pathway of Gq protein-coupled α1B-AR in human osteoblasts.

R. Yamamoto, S. Ueki, Y. Moritoki, Y. Kobayashi, H. Oyamada,Y. Konno, M. Tamaki, M. Itoga, M. Takeda, W. Ito and J. Chihara. **Adiponectin attenuates human eosinophil adhesion and chemotaxis: implications in allergic inflammation.** *J Asthma* 2013, 50(8), 828-35 [doi:10.3109/02770903.2013.816725]

Yamamoto et al used Cal-520 AM to monitor the expression of eotaxin receptor CCR3 and intracellular calcium influx by flow cytometry. AdipoR1 and AdipoR2 were expressed in human eosinophils. Adiponectin did not affect eosinophil survival or CCR3 expression while eotaxin-enhanced adhesion was inhibited by pretreatment with adiponectin. Adiponectin also diminished eotaxin-directed chemotactic responses by disturbing both velocity and directionality. Calcium influx in response to eotaxin was attenuated by adiponectin. The authors concluded that adiponectin attenuates the eosinophil functions induced by eotaxin without affecting cell viability. The inhibitory effect was associated with diminished calcium signaling rather than altering of surface receptor expression.

M. Liu, J. Liu; J. Liao, Z. Diwu. **A Functional Analysis of GPCR and Calcium Channel Targets Using Cal 520 AM Ester**. *Biophys J*, 2012, 102(3), 309a-310a [doi:10.1016/j.bpj.2011.11.1706].

Liu et al evaluated the signal intensity and signal to background ratio of Cal 520 AM in several cell lines including HEK-293, CHO-M1 and Jurkat cells with different receptor signaling pathways. Cal 520 AM were demonstrated to have much better cell retention ability in addition to its significantly higher signal to background ratio comparing to Fluo-3 AM and Fluo-4 AM. Cal-520 AM requires minimal amount of probenecid present while Fluo-3 AM and Fluo-4 AM requires a significantly amount of probenecid present to prevent the dyes from leaking out of host cells. This feature enables Cal-520 AM for calcium assays in probenecid-sensitive cell lines. Cal-520 AM was a significantly improved fluorescent indicator for monitoring intracellular calcium. The high signal-to-noise ratio and good intracellular retention properties make the Cal 520 AM a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

California Red™ & SunRed™
Superior Replacements for Texas Red® and Texas Red®-X

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

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

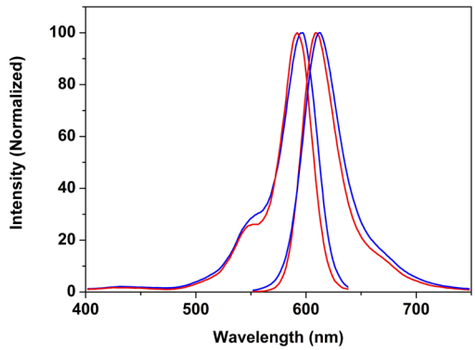


Figure 4.1. Spectral comparison of California Red™ and Texas Red® conjugated to Gly-Gly-Sor-Ser-Arg-Trp (Red: Texas Red®; Blue: California Red™).

Table 4.1 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 4.2 Superior Replacement Dyes for Texas Red®

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @260 nm	CF @280 nm
473	California Red™, SE	5 mg	583	603	100,000	0.456	0.360
480	Sulforhodamine 101 Sulfonyl Chloride [Texas Red®]	10 mg	588	601	100,000	0.456	0.360
472	SunRed™, SE	5 mg	583	603	98,000	0.240	0.366
485	Texas Red® Alkyne *Single Isomer*	5 mg	588	601	95,000	0.456	0.360
484	Texas Red® Azide *Single Isomer*	5 mg	588	601	95,000	0.456	0.360
482	Texas Red® Cadaverine *Single Isomer*	5 mg	582	602	95,000	0.456	0.360
481	Texas Red® Hydrazide *Single Isomer*	5 mg	582	602	95,000	0.456	0.360
483	Texas Red® Maleimide *Single Isomer*	5 mg	588	601	95,000	0.456	0.360

www.aatbio.com • info@aatbio.com
Tel: 800-990-8053 • Fax: 408-733-1304

Click Chemistry Building Blocks

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

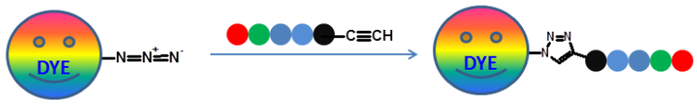


Figure 4.2 The reaction scheme of a biomolecule alkyne with a dye azide.

Table 4.3 Dye Azides for Labeling Oligonucleotides and Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @260 nm	CF @280 nm
508	AMCA Azide	1 mg	353	455	18,000	0.183	0.153
143	Cyanine 3 Azide [equivalent to Cy3® Azide]	1 mg	555	565	150,000	0.042	0.073
153	Cyanine 5 Azide [equivalent to Cy5® Azide]	1 mg	649	665	250,000	0.026	0.030
178	Cyanine 5.5 Azide [equivalent to Cy5.5® Azide]	1 mg	678	701	230,000	0.094	0.101
163	Cyanine 7 Azide [equivalent to Cy7® Azide]	1 mg	749	776	275,000	0.025	0.036
131	5-FAM Azide	10 mg	494	521	75,000	0.247	0.178
133	6-FAM Azide	10 mg	494	521	75,000	0.255	0.172
240	6-Hex Azide	5 mg	533	550	74,000	0.300	0.127
248	6-JOE Azide	5 mg	520	548	73,000	0.326	0.217
494	6-ROX Azide	5 mg	575	602	95,000	0.307	0.179
486	5-TAMRA Azide	5 mg	547	573	75,000	0.288	0.178
490	6-TAMRA Azide	5 mg	547	573	75,000	0.335	0.180
244	6-TET Azide	5 mg	521	536	76,000	0.191	0.104
484	Texas Red® Azide *Single Isomer*	5 mg	588	601	95,000	0.456	0.360
2236	Tide Fluor™ 1 Azide [TF1 Azide]	5 mg	345	442	20,000	0.183	0.187
2252	Tide Fluor™ 2 Azide [TF2 Azide]	1 mg	500	527	75,000	0.288	0.091
2254	Tide Fluor™ 3 Azide [TF3 Azide]	1 mg	555	584	75,000	0.331	0.178
2300	Tide Fluor™ 4 Azide [TF4 Azide]	1 mg	590	618	90,000	0.489	0.436
2275	Tide Fluor™ 5WS Azide [TF5WS Azide]	1 mg	649	664	250,000	0.023	0.027
2302	Tide Fluor™ 6WS Azide [TF6WS Azide]	1 mg	676	695	220,000	0.111	0.101
2304	Tide Fluor™ 7WS Azide [TF7WS Azide]	1 mg	749	775	275,000	0.009	0.049
2188	Tide Quencher™ 1 Azide [TQ1 Azide]	5 mg	515	N/A	20,000	0.147	0.194
2211	Tide Quencher™ 2 Azide [TQ2 Azide]	5 mg	515	N/A	21,000	0.100	0.120
2231	Tide Quencher™ 3 Azide [TQ3 Azide]	5 mg	570	N/A	22,000	0.085	0.091
2068	Tide Quencher™ 4WS Azide [TQ4WS Azide]	1 mg	603	N/A	90,000	0.149	0.136
2082	Tide Quencher™ 5WS Azide [TQ5WS Azide]	1 mg	661	N/A	130,000	0.072	0.082
2097	Tide Quencher™ 6WS Azide [TQ6WS Azide]	1 mg	704	N/A	130,000	0.120	0.102
2112	Tide Quencher™ 7WS Azide [TQ7WS Azide]	1 mg	763	N/A	140,000	0.072	0.091

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 or an oligonucleotide, is incorporated by conventional synthesis. Subsequently, the other reaction partner—the complementary alkyne or azide linked to a fluorescent dye, biotin or other detection reagent—is "clicked" into place in the presence of catalytic copper (I). One reaction partner must be an azide derivative and the other 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 or an oligonucleotide and a dye is extremely stable. It is not susceptible to hydrolysis, oxidation or reduction. AAT Bioquest offers a variety of dye azides and alkynes for labeling peptides and oligonucleotides. 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.

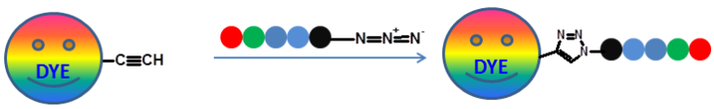


Figure 4.3 The reaction scheme of a biomolecule azide with a dye alkyne.

Table 4.4 Dye Alkynes for Labeling Oligonucleotides and Peptides

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1}M^{-1}$)	CF @260 nm	CF @280 nm
507	AMCA Alkyne	1 mg	353	455	18,000	0.183	0.153
144	Cyanine 3 Alkyne [equivalent to Cy3® Alkyne]	1 mg	555	565	150,000	0.042	0.073
154	Cyanine 5 Alkyne [equivalent to Cy5® Alkyne]	1 mg	649	665	250,000	0.026	0.030
179	Cyanine 5.5 Alkyne [equivalent to Cy5.5® Alkyne]	1 mg	678	701	230,000	0.094	0.101
164	Cyanine 7 Alkyne [equivalent to Cy7® Alkyne]	1 mg	749	776	275,000	0.025	0.036
132	5-FAM Alkyne	10 mg	494	521	75,000	0.247	0.178
134	6-FAM Alkyne	10 mg	494	521	75,000	0.255	0.172
241	6-HEX Alkyne	5 mg	533	550	74,000	0.300	0.127
249	6-JOE Alkyne	5 mg	520	548	73,000	0.326	0.217
495	6-ROX Alkyne	5 mg	575	602	95,000	0.307	0.179
487	5-TAMRA Alkyne	5 mg	547	573	75,000	0.288	0.178
491	6-TAMRA Alkyne	5 mg	547	573	75,000	0.335	0.180
245	6-TET Alkyne	5 mg	521	536	76,000	0.191	0.104
485	Texas Red® Alkyne *Single Isomer*	5 mg	588	601	95,000	0.456	0.360
2237	Tide Fluor™ 1 Alkyne [TF1 Alkyne]	5 mg	345	442	20,000	0.183	0.187
2253	Tide Fluor™ 2 Alkyne [TF2 Alkyne]	1 mg	500	527	75,000	0.288	0.091
2255	Tide Fluor™ 3 Alkyne [TF3 Alkyne]	1 mg	555	584	75,000	0.331	0.178
2301	Tide Fluor™ 4 Alkyne [TF4 Alkyne]	1 mg	590	618	90,000	0.489	0.436
2276	Tide Fluor™ 5WS Alkyne [TF5WS Alkyne]	1 mg	649	664	250,000	0.023	0.027
2303	Tide Fluor™ 6WS Alkyne [TF6WS Alkyne]	1 mg	676	695	220,000	0.111	0.101
2305	Tide Fluor™ 7WS Alkyne [TF7WS Alkyne]	1 mg	749	775	275,000	0.009	0.049
2189	Tide Quencher™ 1 Alkyne [TQ1 Alkyne]	5 mg	515	N/A	20,000	0.147	0.194
2212	Tide Quencher™ 2 Alkyne [TQ2 Alkyne]	5 mg	515	N/A	21,000	0.100	0.120
2232	Tide Quencher™ 3 Alkyne [TQ3 Alkyne]	5 mg	570	N/A	22,000	0.085	0.091
2069	Tide Quencher™ 4WS Alkyne [TQ4WS Alkyne]	1 mg	603	N/A	90,000	0.149	0.130
2083	Tide Quencher™ 5WS Alkyne [TQ5WS Alkyne]	1 mg	661	N/A	130,000	0.072	0.082
2098	Tide Quencher™ 6WS Alkyne [TQ6WS Alkyne]	1 mg	704	N/A	130,000	0.120	0.102
2113	Tide Quencher™ 7WS Alkyne [TQ7WS Alkyne]	1 mg	763	N/A	140,000	0.072	0.091

International Distributors

Austria:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Australia:
Life Research Pty Ltd.
Email: info@liferesearch.com
Website: http://www.liferesearch.com

Belgium:
Gentaur BVBA
Email: info@gentaur.com
Website: http://www.gentaur.com

Canada:
Cedarlane Laboratories Ltd.
Email: sales@cedarlanelabs.com
Website: http://www.cedarlanelabs.com

China:
AmyJet Scientific Inc.
Email: amyjetsci@gmail.com
Website: http://www.amyjet.com

Dakewe Biotech Co., Ltd
Email: sale_shenzhen@dakewe.net
Website: http://www.dakewe.net

Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Tianjin Biolite Biotech Co., Ltd
Email: info@tjbiolite.com
Website: http://www.tjbiolite.com

Croatia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Czech Republic:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

Denmark:
Nordic BioSite ApS
Email: info@nordicbiosite.dk
Website: http://www.nordicbiosite.dk

Estonia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Finland:
Nordic BioSite OY
Email: info@biosite.fi
Website: http://www.biosite.fi

France:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Interchim
Email: [illegible]
Website: http://www.interchim.com

Germany:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Hong Kong:
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Hungary:
IZINTA Trading Co., Ltd.
Email: baloghk@izinta.hu
Website: http://www.izinta.hu

Iceland:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

India:
Biochem Life Sciences
Email: info@bcls.in
Website: http://www.bcls.in

GenxBio Health Sciences Pvt. Ltd,
Email: sales@genxbio.com
Email: genxbio@gmail.com
Website: http://www.genxbio.com

Ireland:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

Israel:
ADVANSYS Technologies for Life Ltd.
Email: info@advansys.co.il
Website: http://www.advansys.co.il

Italy:
Space Import Export S.r.l.
Email: info@spacesrl.com
Website: http://www.spacesrl.com

Valter Occhiena S.r.l.
Email: vo@valterocchiena.com
Website: http://www.valterocchiena.com

Japan:
Cosmo Bio Co., Ltd.
Email: mail@cosmobio.co.jp
Website: http://www.cosmobio.co.jp

Nacalai Tesque, Inc.
Email: info@nacalaiusa.com
Website: http://www.nacalai.com

Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

Korea:
Cheong Myung Science Corporation
Email: cms@cmscorp.co.kr
Website: http://www.cmscorp.co.kr

Latvia and Lithuania:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Poland and Slovenia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Romania:
SC VitroBioChem SRL
Email: office@vitro.ro
Website: http://www.vitro.ro

Singapore and Other South Asian Countries:
BST Scientific Pte Ltd.
Email: info@bstsci.com
Website: http://www.bstsci.com

Slovakia:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

South American Countries and Regions:
Impex Comércio Internacional Ltda.
Email: impexcom@terra.com.br
Website: http://www.impexbrasil.com.br

Spain and Portugal:
Deltaclon S. L
Email: info@deltaclon.com
Website: http://www.deltaclon.com

Sweden:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Switzerland:
LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Taiwan:
Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Turkey:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

AAT Bioquest®
Advancing Assay & Test Technologies