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AssayWise Letters

New Product Highlights

LysoBrite™ lysosome stains
Multiplexing apoptosis, necrosis and caspases

Cell-Based Assays

Cal-520™ for Ca²⁺ detection

Biochemical Assays

NAD/NADH and NADP/NADPH detection

Drug Discovery Tools

Fluo-8® for Ca²⁺ detection
cAMP detection in live cells

Fluorescent Labeling Probes

Bright iFluor™ labeling dyes
Rapid ReadLink™ labeling kits

Bioprocess Tools

Protease, thiol and Ca²⁺ detection

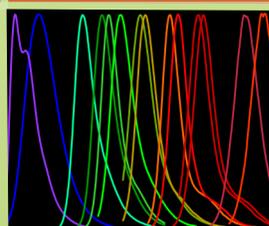
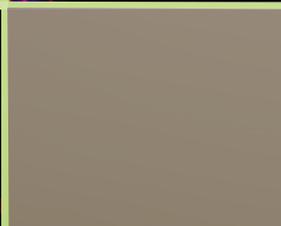
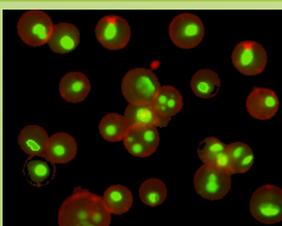
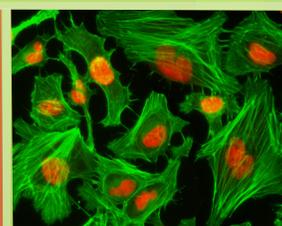
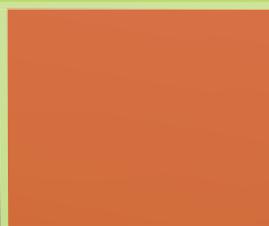
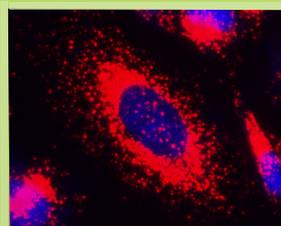


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From the President of AAT Bioquest

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 launching 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

Trademarks of AAT Bioquest

AAT Bioquest®	LysoBrite™
Amplite™	mFluor™
Apoptin™	Nuclear Green™
Calcein Green™	Nuclear Orange™
Calcein Orange™	Nuclear Red™
Calcein Red™	ProRed™
Cell Explorer™	ReadILink™
Cell Navigator™	Screen Quest™
Cell Meter™	Thiolite™
CytoCalcein™	Tide Fluor™
Fluo-8®	Tide Quencher™
iFluor™	VFSE™

Trademarks of Other Companies

Alexa Fluor® (Invitrogen)	LysoTracker® (Invitrogen)
Cy3®, Cy5®, Cy7® (GE Healthcare)	Texas Red® (Invitrogen)

Light up Lysosomes with Cell Navigator™ Kits

Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The interior of the lysosome is acidic (pH 4.5-4.8) compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels.

AAT Bioquest Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

Our Cell Navigator™ Lysosome Staining Kits are designed to label lysosomes of live cells with LysoBrite™ dyes, our proprietary lysotropic indicators which selectively accumulate in lysosomes probably via the lysosome pH gradient. The LysoBrite™ dyes are hydrophobic compounds that easily permeate intact live cells, and trapped in lysosomes after they get into cells. Their fluorescence is significantly enhanced upon entering lysosomes. This key feature significantly reduces the staining background and makes the assay kits useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The Cell Navigator™ staining kits are suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. The labeling protocols are robust, requiring minimal hands-on time. The kits can be readily adapted for many types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope.

Key Features of Cell Navigator™ Staining Kits:

- **Minimal cytotoxicity**, no cell toxicity observed.
- **Multiplexing wavelengths**, Ex/Em = 450/505 nm, 542/556 nm, 575/597 nm and 596/619 nm.
- **Increased signal intensity**, 10 times brighter than LysoTracker® dyes (Invitrogen).
- **Extraordinarily high photostability**, no fading observed with 2 minutes exposure.
- **Excellent cellular retention**, more than 6 passages for cell tracking in HeLa cells.
- **Fixable**, cell staining pattern survives fixation.

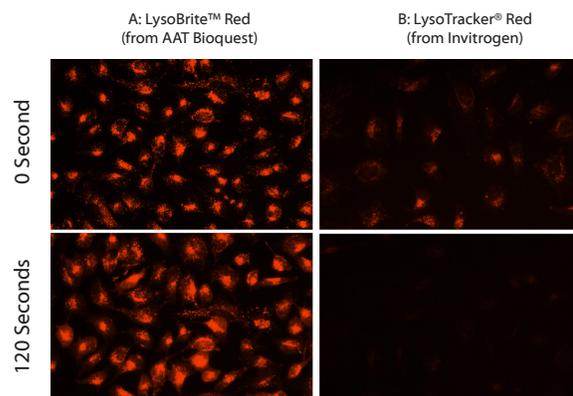


Figure 1.1. Images of HeLa cells stained with A: Cell Navigator™ Lysosome Staining Kit, B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar blackwall/clear bottom 96-well plate. The signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

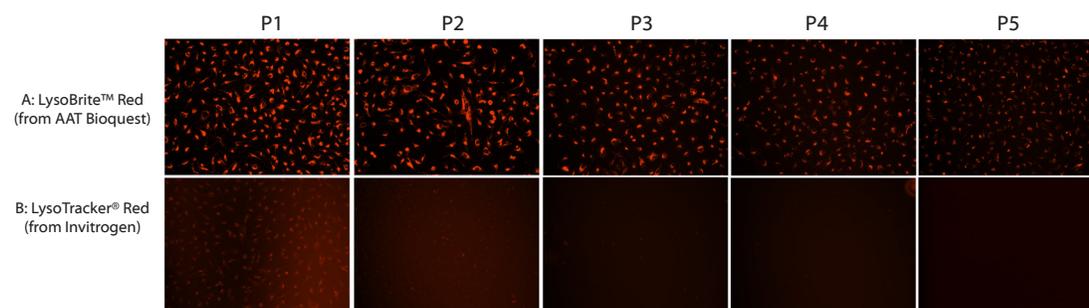


Figure 1.2. Images of HeLa cells stained with A: Cell Navigator Lysosome Staining Kit (Top, from AAT Bioquest), B: LysoTracker® Red DND-99 (Bottom, from Invitrogen) in a Costar black wall/clear bottom 96-well plate. The signals were compared at 5 cell passages (P1, P2, P3, P4 and P5) respectively using an Olympus fluorescence microscope.

Table 1.1. Cell Navigator™ Assay Kits for Staining Lysosomes in Live Cells

Cat. #	Product Description	Ex (nm)	Em (nm)	Size	Price
22656	Cell Navigator™ Lysosome Staining Kit *Green Fluorescence*	450	505	500 Assays	\$145
22657	Cell Navigator™ Lysosome Staining Kit *Orange Fluorescence*	542	556	500 Assays	\$145
22658	Cell Navigator™ Lysosome Staining Kit *Red Fluorescence*	575	597	500 Assays	\$145
22659	Cell Navigator™ Lysosome Staining Kit *Deep Red Fluorescence*	596	619	500 Assays	\$145

Multiplex Apoptosis and Necrosis

Apoptosis is an active, programmed process of autonomous cellular dismantling that avoids eliciting inflammation. In apoptosis, phosphatidylserine (PS) is transferred to the outer leaflet of the plasma membrane. As a universal indicator of the initial/intermediate stages of cell apoptosis, the appearance of phosphatidylserine on the cell surface can be detected before morphological changes are observed.

Necrosis is characterized as a passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents. Loss of plasma membrane integrity represents a straightforward approach to demonstrate late stage apoptosis and necrosis.

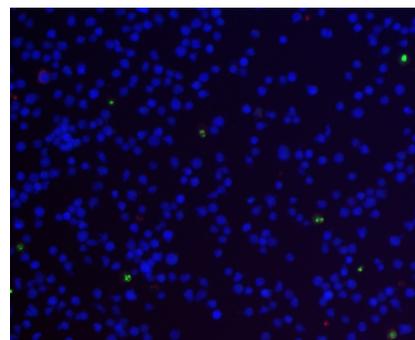
AAT Bioquest offers Cell Meter™ Apoptotic and Necrotic Detection Kits as a set of tools for monitoring cell viability. Our Cell Meter™ detection kits are optimized to simultaneously detect cell apoptosis, necrosis and healthy cells with a flow cytometer or fluorescence microscope. The phosphatidylserine (PS) sensor used in Kit 22843 has deep red fluorescence (Ex/Em = 630/660 nm) upon binding to membrane PS. Membrane-impermeable Nuclear Green™ DCS1 (Ex/Em = 490/525 nm) is used to label the nucleus while CytoCalcein™ Violet 450 (Ex/Em = 405/450 nm) is provided for labeling live cell cytoplasm.

Key Features of Cell Meter™ Detection Kits:

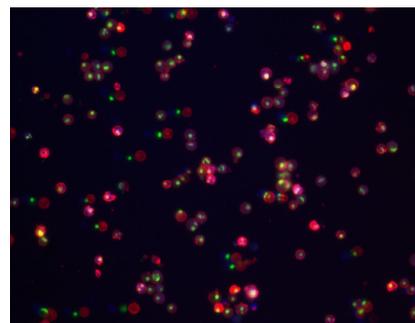
- **Multiplexing capability**, triple colors for the simultaneous detection of multiple cellular events.
- **Robust**, a mix and read format.
- **Convenient**, compatible with common filter sets.

JC-10, a Superior Replacement to JC-1

- ♦ **More water soluble**, DMSO stock solution of JC-10 can be readily diluted into a variety of aqueous buffers.
- ♦ **More sensitive**, detect smaller mitochondrial membrane potential changes in certain cell lines.



A. Live Cells



B. Apoptotic Cells

Figure 1.3. The detection of binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence images demonstrated Jurkat cells that are live (blue, stained by CytoCalcein™ Violet 450), apoptotic (red, stained by Apopxin™ Deep Red), and necrotic (green, indicated by Nuclear Green™ DCS1 staining). The cells were induced by 1 μM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope through the Violet, Cy5 and FITC channels respectively. Individual images were taken from each channel for the same cell population. The images were merged as shown above. A: Non-induced control cells; B: Triple staining of staurosporine-induced cells.

Table 1.2. Cell Meter™ Assay Kits for Detecting Apoptosis and Necrosis

Cat. #	Product Description	Ex (nm)	Em (nm)	Size	Price
22840	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence Color*	405	450	100 Assays	\$245
		490	525		
		546	647		
22843	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence Color*	405	450	100 Assays	\$295
		490	525		
		630	660		
22811	Cell Meter™ Nuclear Apoptosis Assay Kit *Green Fluorescence*	503	526	100 Assays	\$195
22844	Cell Meter™ TUNEL Apoptosis Assay Kit	556	579	50 Assays	\$295
13433	Z-DEVD-ProRed™ 620 *Red Caspase 3/7 Substrate*	534	619	1 mg	\$295

Multiplex Caspase Activities

Caspases, cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases, are a family of cysteine proteases that play essential roles in apoptosis (programmed cell death), necrosis, and inflammation. Caspases are essential in cells for development and most other stages of adult life, and have been termed “executioner” proteins for their roles in cells. Some caspases are also required in the immune system for the maturation of lymphocytes. Failure of apoptosis is one of the main contributions to tumour development and autoimmune diseases. It is also reported that the unwanted apoptosis occurs with ischemia or Alzheimer’s disease. The new findings have stimulated interest in caspases as potential therapeutic targets since they were discovered in the mid-1990s.

Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit (Cat. #22797) is designed to monitor cell apoptosis through measuring Caspase 3 activation. Caspase 3 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase-3 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). Z-DEVD-ProRed™ is used in the kit as the fluorogenic indicator for caspase-3 activity. Cleavage of ProRed™ DEVD blocking peptide residue by caspase 3 generates strongly red fluorescent ProRed™ that is fluorimetrically monitored at ~620 nm with excitation at ~530 nm.

Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit (Cat. #22797) provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput assays in a wide variety of fluorescence platforms such as microplate assays. Using 100 μ L of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 100 assays. Using 25 μ L of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 400 assays.

Key Features of Cell Meter™ Caspase 3/7 Activity Assay Kits:

- **Multicolor capability**, tricolor panel enables the simultaneous detection of multiple caspase activations (see Figure 1.5).
- **Robust**, a mix and read format.
- **Convenient**, compatible with common filter sets.

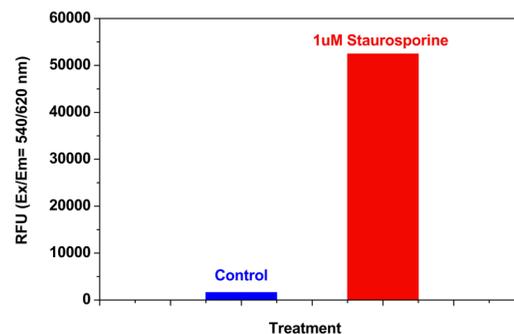


Figure 1.4. Detection of caspase 3/7 activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells /well/90 μ L in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 μ M staurosporine for 5 hours. The caspase 3/7 assay solution (100 μ L/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 540/620 nm.

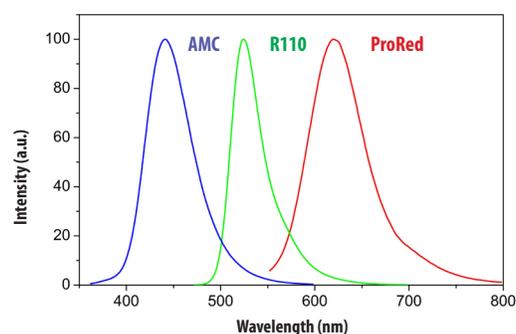


Figure 1.5. The fluorescence spectra of AMC, R110 and ProRed™ in aqueous buffer (pH 7.0). AMC, R110 and ProRed™ caspase substrates are well suited for multiplexing caspase activities.

Table 1.3. Cell Meter™ Assay Kits for Detecting Caspase Activity

Cat. #	Product Description	Size	Price
22795	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 Assays	\$95
22796	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Green Fluorescence*	200 Assays	\$195
22823	Cell Meter™ Caspase 3/7 Activity Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 Assays	\$195
22797	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Red Fluorescence*	100 Assays	\$295
22812	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 Assays	\$195
22798	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Green Fluorescence*	200 Assays	\$195
22816	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Red Fluorescence*	100 Assays	\$295
22813	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 Assays	\$195
22799	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Green Fluorescence*	200 Assays	\$295

Cal-520™, the Best Green Fluorescent Ca²⁺ Dye

Cal-520™ AM is our newest fluorogenic calcium-sensitive dye with a significantly improved signal to noise ratio and intracellular retention compared to the existing green calcium indicators (such as Fluo-3 AM and Fluo-4 AM). Cal-520™ provides a robust homogeneous fluorescence-based assay tool for detecting intracellular calcium mobilization.

Cells expressing a GPCR or calcium channel of interest that signals through calcium can be preloaded with Cal-520™ AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Cal 520™ AM are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells. Its fluorescence is greatly enhanced upon binding

to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increases the fluorescence of Cal-520™.

The characteristics of its excellent cellular retention, high sensitivity and >100 times fluorescence enhancement make Cal-520™ AM an ideal indicator for the measurement of intracellular calcium. The high S/N ratio and better intracellular retention make the Cal-520™ calcium assay the most robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists. This new indicator has been successfully used for probenecid-sensitive cell lines and drug discovery targets.

Key Features of Cal-520™ Calcium Indicators (compared to Fluo-4):

- **Excellent cellular retention**, enabling Ca²⁺ assays of probenecid-sensitive GPCRs and Ca²⁺ channels.
- **Robust**, significantly higher S/N ratio than those of Fluo-4 AM and any other commercially available fluorescent Ca²⁺ assays.
- **Convenient**, essentially identical spectra to those of Fluo-4 and Fluo-8®.
- **Versatile packing sizes to meet your special needs**, 1 mg; 10x50 µg; HTS packages.

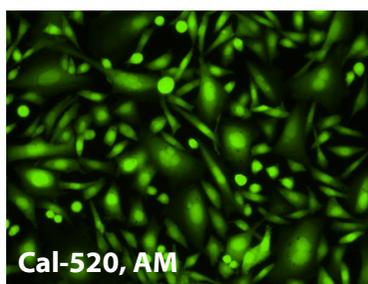
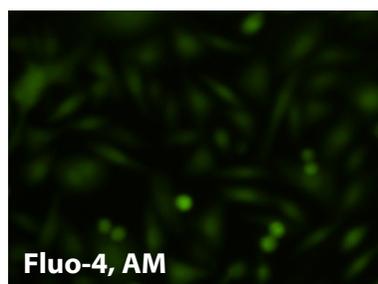


Figure 2.1. Responses of endogenous P2Y receptor to ATP in CHO-M1 cells without probenecid. CHO-M1 cells were seeded overnight at 40,000 cells per 100 µL per well in a Costar 96-well black wall/clear bottom plate. 100 µL of 4 µM Fluo-4 AM (left), Cal 520™ AM (right) in HHBS was added into the wells, 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.

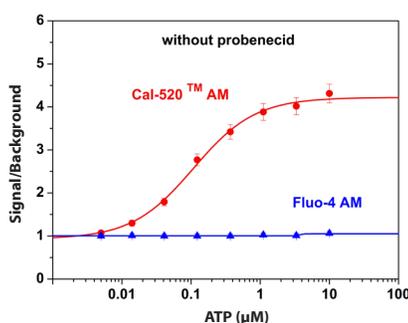
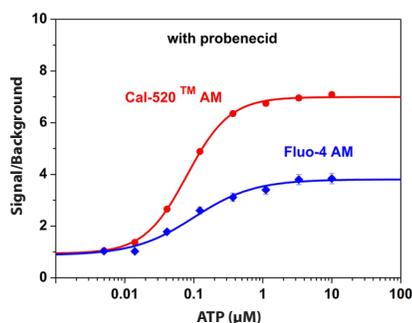


Figure 2.2. ATP-stimulated calcium responses of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-520™ AM (red curve), or Fluo-4 AM (blue curve) respectively with (left) or without probenecid (right) under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100 µL per 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 FlexSation to achieve the final indicated concentrations.

Table 2.1. Spectral and Ca²⁺-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

Table 2.2. The Best Value of Cal-520™ for Calcium Detection

Ca ²⁺ Indicator	AAT Bioquest	Sigma [†]	Invitrogen [†]
Cal-520™ AM	\$395/mg (\$198/mg [‡])	not available	not available
Fluo-3 AM	\$145/mg (\$73/mg [‡])	\$390/mg	\$230/mg
Fluo-4 AM	not available	not available	\$412/mg (\$206/10x50 µg)

[†]Prices are based on the websites of Sigma-Aldrich and Invitrogen as of May, 2012 respectively, which may vary for different customers.

[‡]Special 50% introduction discount applied (Discount Code: IDC2012, valid until 12/31/2012).

Table 2.3. Ca²⁺ Detection Reagents and Kits

Cat. #	Product Description	Size	Price
21130	Cal-520™, AM *Cell-permeable*	10x50 µg	\$295
21131	Cal-520™, AM *Cell-permeable*	1 mg	\$395
21135	Cal-520™, sodium salt	10x50 µg	\$295
36336	Cal-520™ Medium Removal Calcium Assay Kit *Optimized for Difficult Cell Lines*	10 Plates	\$495
36337	Cal-520™ Medium Removal Calcium Assay Kit *Optimized for Difficult Cell Lines*	100 Plates	\$3,950
36338	Cal-520™ No-Wash Calcium Assay Kit *Optimized for Difficult Cell Lines*	10 Plates	\$495
36339	Cal-520™ No-Wash Calcium Assay Kit *Optimized for Difficult Cell Lines*	100 Plates	\$3,950
36400	Cal-520™ PBX Calcium Assay Kit *Optimized for Probenecid-Sensitive Cell Lines*	10 Plates	\$695
36401	Cal-520™ PBX Calcium Assay Kit *Optimized for Probenecid-Sensitive Cell Lines*	100 Plates	\$4,950
21143	Cal-520FF™, AM	10x50 µg	\$345
21144	Cal-520FF™, potassium salt	10x50 µg	\$345
21011	Fluo-3, AM *UltraPure grade*	1 mg	\$175
21014	Fluo-3 FF, AM *UltraPure grade*	10x50 µg	\$195
21019	Fluo-3 FF, pentapotassium salt	1 mg	\$295
21021	Fluo-2, AM *UltraPure grade*	1 mg	\$95
21032	Indo-1, AM *UltraPure Grade*	1 mg	\$145
21080	Fluo-8 [®] , AM *Cell-permeable*	1 mg	\$245
21090	Fluo-8H™, AM *Cell-permeable*	1 mg	\$295
21097	Fluo-8L™, AM *Cell-permeable*	10x50 µg	\$195
21104	Fluo-8FF™, AM *Cell-permeable*	10x50 µg	\$195
21120	Rhod-4™, AM *Cell-permeable*	1 mg	\$495
21062	Rhod-2, AM *UltraPure grade*	1 mg	\$195
21072	Rhod-5N, tripotassium salt	1 mg	\$245
21077	Rhod-FF, AM *Cell-permeable*	1 mg	\$295
36315	Screen Quest™ No-Wash Calcium Assay Kit	10 Plates	\$295
36308	Screen Quest™ Medium Removal Calcium Assay Kit	10 Plates	\$245

The Rapid & Sensitive Detection of NAD, NADH, NADP & NADPH Using Amplite™ Assay Kits

NAD, NADH, NADP and NADPH are important cofactors in cells. AAT Bioquest Amplite™ NAD(P)/NAD(P)H assay kits have very low background since they are performed in a longer wavelength range that considerably reduces the interference resulted from biological samples. They have demonstrated high selectivity and enhanced sensitivity. Our kits enable the detection of NAD, NADH, NADP & NADPH by either using fluorimetric assay mode or colorimetric assay mode. These kits have been used for :

- Studying cell metabolism
- Measuring NAD(H) and NADP(H) in clinical research samples
- Screening activities of enzymes (such as a variety of dehydrogenases and reductases).

Key Features of Amplite™ Assay Kits:

- **Broad applications**, can be used for quantifying NAD(P)/NAD(P)H in solutions and in cell extracts.
- **Sensitive**, detect as low as 1 μM of NAD(P)/NAD(P)H.
- **Convenient**, no wash is required.
- **Non-radioactive**, no special requirements for waste treatment.

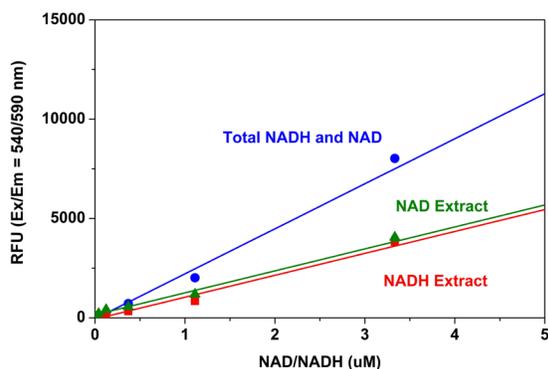


Figure 3.1. Total NAD/NADH, and their extract dose responses were measured with Amplite™ NAD/NADH Ratio Assay Kit in a black 96-well plate using a Gemini microplate reader (Molecular Devices).

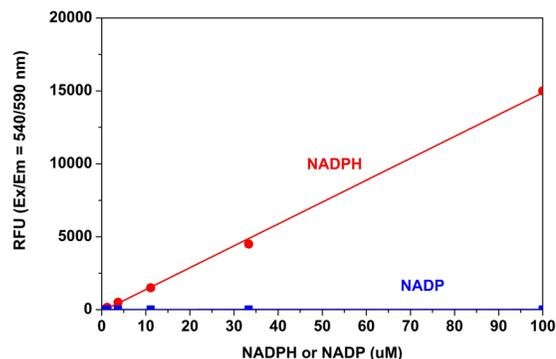


Figure 3.2. NADPH/NADP dose responses were measured with Amplite™ NADPH Assay Kit in a black 96-well plate using a NOVOstar microplate reader (BMG Labtech). As low as 1 μM (100 pmol/well) of NADPH can be detected with 1 hour incubation (n=3) while there is no response from NADP.

Table 3.1. Assay Kits for NAD/NADH Detection

Cat. #	Product Description	Size	Price
15258	Amplite™ Colorimetric NAD/NADH Assay Kit	400 Assays	\$195
15261	Amplite™ Fluorimetric NADH Assay Kit	400 Assays	\$195
15257	Amplite™ Fluorimetric NAD/NADH Assay Kit	400 Assays	\$295
15263	Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit	250 Assays	\$345

Table 3.2. Assay Kits for NADP/NADPH Detection

Cat. #	Product Description	Size	Price
15260	Amplite™ Colorimetric NADP/NADPH Assay Kit	400 Assays	\$195
15262	Amplite™ Fluorimetric NADPH Assay Kit	400 Assays	\$195
15259	Amplite™ Fluorimetric NADP/NADPH Assay Kit	400 Assays	\$295
15264	Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit	250 Assays	\$345

Quantify Maleimide by Absorbance or Fluorescence

Maleimides can be directly assayed spectrophotometrically at 302 nm. However, the small extinction coefficient of $620 \text{ M}^{-1}\text{cm}^{-1}$ renders this assay insensitive, and the assay is further complicated by the protein absorbance at the same wavelength. Although the enzyme-based maleimide quantification is more sensitive, the method is expensive and extremely time-consuming.

Our Amplite™ Colorimetric Maleimide Assay Kit quantifies maleimide groups by first reacting a sample with a known amount of thiol present in excess and then assaying the remaining unreacted thiol using 4,4'-DTDP with a molar extinction coefficient of $19,800 \text{ M}^{-1}\text{cm}^{-1}$. The amount of maleimide is calculated as the difference between the initial amount of thiol and the amount of unreacted thiol after the complete reaction of all maleimide groups. This spectrophotometric assay for the determination of maleimide groups is a reverse GSH assay. It takes advantage of the high reactivity of thiols of GSH with the maleimide moiety. Maleimide of the sample is allowed to form a stable thiosuccinimidyl linkage with GSH. After the reaction is complete, the excess GSH, i.e., the remaining thiols of GSH in the reaction mixture, is estimated by using 4,4'-DTDP. The amount of GSH reacted with the sample is titrated to determine the extent of maleimide.

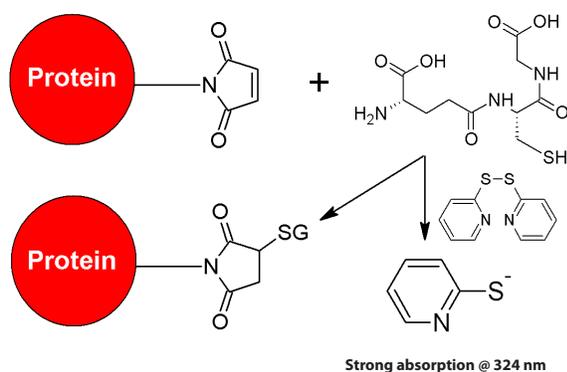


Figure 3.3. 4,4'-DTDP assay principle for quantifying maleimide

Sensitive assays of maleimide and thiol groups are required for the efficient conjugation of proteins that are expensive and available only in small amounts. A variety of crosslinking reagents with a maleimide group are widely used for crosslinking proteins to proteins or proteins to other biomolecules. There are few reagents or assay kits available for quantifying the number of maleimide groups that are introduced into the first protein. All the commercial kits have tedious protocols.

Our Amplite™ Fluorimetric Maleimide Quantitation Kit uses a proprietary dye that has enhanced fluorescence upon reacting with a maleimide. The kit provides a sensitive, one-step fluorimetric method to detect as little as 10 picomoles of maleimide in a 100 μL assay volume (100 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader at $\text{Ex/Em} = 490/525 \text{ nm}$. Compared to kit 5525, this fluorometric assay is more sensitive, and has less interference from biological samples.

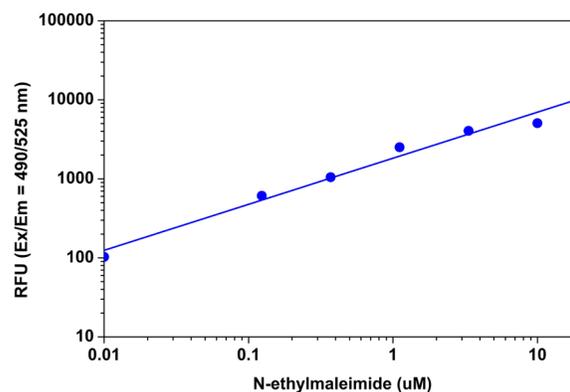


Figure 3.4. N-ethylmaleimide dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 0.1 μM (10 pmol/well) of maleimide was detected with 10 minutes incubation ($n=3$).

Key Features of Amplite™ Maleimide Quantitation Kits:

- **Broad applications**, quantify maleimide groups in a variety of molecules.
- **Sensitive**, detect as low as 10 picomoles of maleimide (Cat. # 5523).
- **Continuous**, easily adapted to automation without a separation step.
- **Convenient**, formulated to have minimal hands-on time. No wash is required.
- **Non-radioactive**, no special requirements for waste treatment.

Table 3.3. Product Ordering Information

Cat. #	Product Description	Size	Price
5525	Amplite™ Colorimetric Maleimide Quantitation Kit	100 Assays	\$195
5523	Amplite™ Fluorimetric Maleimide Quantitation Kit	200 Assays	\$195

Fluo-8[®], the Brightest Green Ca²⁺ Indicators

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding Ca²⁺ have enabled researchers to investigate changes in intracellular free Ca²⁺ concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Fluo-3 and Fluo-4 are most commonly used among the visible light-excitable calcium indicators. However, Fluo-3 AM and Fluo-4 AM are only moderately fluorescent in live cells upon esterase hydrolysis, and require harsh cell loading conditions to maximize their cellular calcium responses.

It's time to update your tools without additional cost!

Fluo-8[®] has been developed to improve cell loading and calcium response while maintaining the convenient Fluo-3 and Fluo-4 spectral wavelength of maximum excitation

@ ~490 nm and maximum emission @ ~520 nm. Fluo-8[®] AM only requires room temperature while Fluo-3 AM and Fluo-4 AM require 37 °C cell loading. In addition, Fluo-8[®] is 2 times brighter than Fluo-4 AM, and 4 times brighter than Fluo-3 AM.

AAT Bioquest offers a set of our outstanding Fluo-8[®] reagents with different calcium binding affinities (Fluo-8[®]: K_d = 389 nM; Fluo-8H[™]: K_d = 232 nM; Fluo-8L[™]: K_d = 1.86 μM; Fluo-8FF[™]: K_d = 10 μM). We also offer versatile packing sizes to meet your special needs, e.g., 1 mg, 10x50 μg or 20x50 μg.

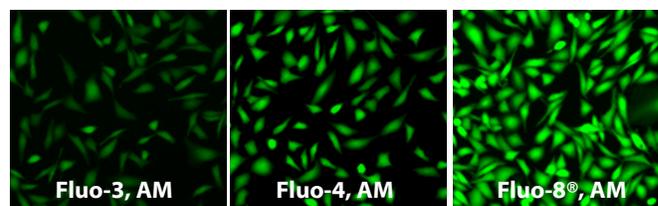


Figure 4.1. U2OS cells were seeded overnight at 40,000 cells per 100 μL per well in a Costar black wall/clear bottom 96-well plate. The growth medium was removed, and the cells were incubated with 100 μL of 4 μM Fluo-3 AM, Fluo-4 AM and Fluo-8[®] AM in HHBS at 37 °C for 1 hour. The cells were washed twice with 200 μL HHBS, then imaged with a fluorescence microscope using FITC channel.

Table 4.1. Fluo-8[®] Reagents with Different Calcium Binding Affinities

Cat. #	Product Description	K _d (Ca ²⁺ -Binding)	Size	Price
21080	Fluo-8 [®] , AM	389 nM	1 mg	\$245
21081	Fluo-8 [®] , AM	389 nM	5x50 μg	\$95
21082	Fluo-8 [®] , AM	389 nM	10x50 μg	\$175
21083	Fluo-8 [®] , AM	389 nM	20x50 μg	\$295
21090	Fluo-8H [™] , AM	232 nM	1 mg	\$295
21091	Fluo-8H [™] , AM	232 nM	10x50 μg	\$195
21096	Fluo-8L [™] , AM	1.86 μM	1 mg	\$295
21097	Fluo-8L [™] , AM	1.86 μM	10x50 μg	\$195
21104	Fluo-8FF [™] , AM	10 μM	10x50 μg	\$195
21105	Fluo-8FF [™] , AM	10 μM	1 mg	\$295

Table 4.2. Fluo-8[®] Calcium Assay Kits Available in Different Sizes for Your Convenience

Cat. #	Product Description	Size	Price
36307	Screen Quest [™] Fluo-8 [®] Medium Removal Calcium Assay Kit	1 Plate	\$75
36308	Screen Quest [™] Fluo-8 [®] Medium Removal Calcium Assay Kit	10 Plates	\$245
36309	Screen Quest [™] Fluo-8 [®] Medium Removal Calcium Assay Kit	100 Plates	\$1,950
36314	Screen Quest [™] Fluo-8 [®] No Wash Calcium Assay Kit	1 Plate	\$95
36315	Screen Quest [™] Fluo-8 [®] No Wash Calcium Assay Kit	10 Plates	\$295
36316	Screen Quest [™] Fluo-8 [®] No Wash Calcium Assay Kit	100 Plates	\$2,450

Quantify cAMP by Absorbance or Fluorescence

Cyclic adenosine monophosphate (cAMP) is an important second messenger in many biological processes. cAMP is derived from adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway. cAMP is synthesised from ATP by adenylyl cyclase located on the inner side of the plasma membrane. Adenylyl cyclase is activated by a range of signaling molecules through the activation of adenylyl cyclase stimulatory G (Gs)-protein-coupled receptors and inhibited by agonists of adenylyl cyclase inhibitory G (Gi)-protein-coupled receptors. Liver adenylyl cyclase responds more strongly to glucagon, and muscle adenylyl cyclase responds more strongly to adrenaline.

Colorimetric ELISA cAMP Assay

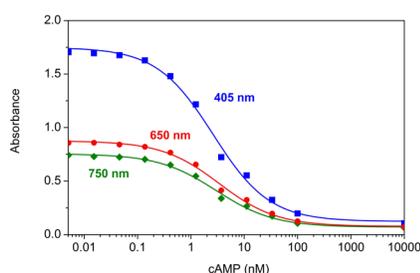


Figure 4.2. cAMP dose response was measured with Screen Quest™ Colorimetric ELISA cAMP Assay Kit in a clear 96-well plate with a SpectraMax microplate reader (Molecular devices). As low as 0.1 nM cAMP can be detected in a 100 µL reaction volume at 405, 650 and 750 nm.

Screen Quest™ Colorimetric ELISA cAMP Assay kit is based on the competition between HRP-labeled cAMP and non-labeled cAMP. HRP-cAMP is displaced from the HRP-cAMP/anti-cAMP antibody complex by unlabeled free cAMP. In the absence of cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. However, the unlabeled free cAMP in the test sample competes for anti-cAMP antibody with the HRP-cAMP antibody conjugate, therefore inhibits the binding of HRP-cAMP to anti-cAMP antibody.

Screen Quest™ Colorimetric ELISA cAMP Assay Kit provides a sensitive method for detecting adenylyl cyclase activity in biochemical or cell-based assay system. Compared to other ELISA cAMP assay

kits, our kit eliminates the tedious acetylation step. The kit uses Amplite™ Green as a colorimetric substrate to quantify the HRP activity. The assay can be performed in a convenient 96-well plate.

Fluorimetric ELISA cAMP Assay

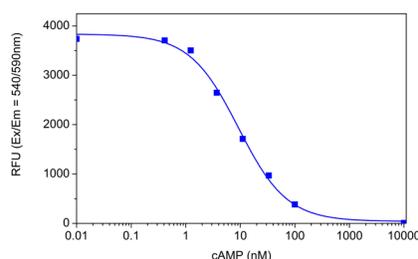


Figure 4.3. cAMP dose response was measured with Screen Quest™ Fluorimetric ELISA cAMP Assay Kit in a solid black 96-well plate with a Gemini microplate reader (Molecular Devices). As low as 0.1 nM cAMP was detected in a 100 µL reaction volume.

Screen Quest™ Fluorimetric ELISA cAMP Assay Kit provides a sensitive method for detecting adenylyl cyclase activity. Compared to other commercial ELISA cAMP assay kits, this cAMP assay kit only requires a single wash step to remove unbound material prior to the development step. It also eliminates the tedious acetylation step. The kit uses Amplite™ Red as a fluorogenic HRP substrate to quantify the HRP activity. The fluorescent product formed is proportional to the activity of HRP-cAMP conjugate.

Live Cell cAMP Assay

Screen Quest™ Live Cell cAMP Assay Service Pack provides the real-time monitoring of intracellular cAMP change in a high-throughput format without a cell lysis step. The assay works through the cell lines that contain either an exogenous cyclic nucleotide-gated channel (CNGC) or the promiscuous G-protein, G_{α16}. The channel is activated by elevated levels of intracellular cAMP, resulting in ion flux and cell membrane depolarization which can be detected with a fluorescent calcium (such as Fluo-8® AM and Cal-520™ AM). Co-expression of G_{α16} with specific non-G_q-coupled receptors will result in the generation of an intracellular calcium signal upon receptor stimulation. The Screen Quest™ Live Cell cAMP Assay Service Pack provides both cell lines and reagents.

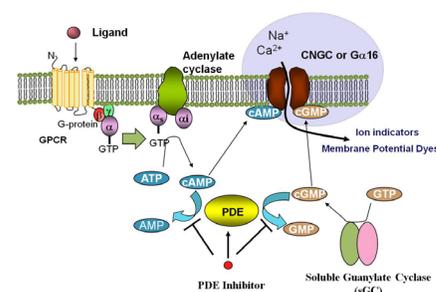


Figure 4.4. Screen Quest™ Live Cell cAMP Assay Principle.

Table 4.3. Screen Quest™ cAMP Assay Kits

Cat. #	Product Description	Size	Price
36370	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	1 Plate	\$295
36371	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	10 Plates	\$1,950
36373	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	1 Plate	\$295
36374	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	10 Plate	\$1,950
36382	Screen Quest™ Live Cell cAMP Assay Servie Pack	Each	Inquire

Optimized Fluorescence Labeling Solutions

Fluorescence is the result of a three-stage process that occurs in certain molecules (generally polyaromatic hydrocarbons or heterocycles) called fluorophores or fluorescent dyes. A fluorescent probe is a fluorophore designed to localize within a specific region of a biological specimen or to respond to a specific stimulus. Fluorescent probes enable researchers to detect particular components of complex biomolecular assemblies (including live cells) with exquisite sensitivity and selectivity. In general, the preferred bioconjugates should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules.

There are a number of fluorescent dyes developed and commercialized for labeling biomolecules. Among them FITC might be the most popular one although it has certain limitations, e.g., pH dependence, low photostability and short wavelengths etc. Alexa Fluor® dyes have been used for labeling proteins and other biomolecules with improved properties over the classic dyes such as fluorescein and rhodamine molecules. However, the extraordinary high costs of Alexa Fluor® dyes keep them from the certain applications that require to use a large amount of dyes such as labeling peptides and oligos. In addition, Alexa Fluor® dyes do not provide a significant benefit for labeling peptides, oligo and other small molecules.

AAT Bioquest offers a complete set of fluorescent labeling dyes that are tiered and optimized for a variety of particular applications with significantly reduced cost. Our *iFluor*™ dyes are a series of excellent fluorescent labeling dyes that span the full UV-visible spectrum. *iFluor*™ dyes are optimized for labeling antibodies and other biopolymers including nucleic acids and carbohydrates. They demonstrate strong fluorescence, high photostability and pH independence on proteins and other biopolymers. All the *iFluor*™ dyes have excellent water solubility. Their hydrophilic property makes the protein conjugation to be readily performed in aqueous media, minimizing the use

of organic solvents. The resulting conjugates are resistant to precipitation during storage. The *iFluor*™ dyes also have much better labeling performance than the classic fluorescent labeling dyes such as FITC, TRITC, Texas Red®, Cy3®, Cy5® and Cy7®. Some of our *iFluor*™ dyes significantly outperform Alexa Fluor® labeling dyes on certain antibodies.

iFluor™ dyes are the products of our recent R&D efforts. AAT Bioquest is rapidly expanding our product lines to meet your constantly changing research needs. We have been developing dyes to solve various limitations with the existing fluorescent labeling reagents while offering classic fluorescent labeling reagents which have high purity and competitive pricing to help you to get more research done with less fund.

Key Features of *iFluor*™ Dyes:

- **Versatile**, available in a variety of reactive forms.
- **Bright**, *iFluor*™ conjugates exhibit more intense fluorescence than other spectrally similar conjugates of classic fluorescent dyes such as FITC, TAMRA and ROX under similar excitations.
- **Photostable**, *iFluor*™ dyes are more photostable than the classic fluorescent dyes such as FITC, Cy3® and Cy5®.
- **Well excited**, *iFluor*™ dyes have absorption spectra that match the principal output wavelengths of common excitation sources (such as 488 nm, 555 nm, 633 nm and 647 nm).
- **Multicolor**, *iFluor*™ dyes and their conjugates are available in a variety of distinct fluorescent colors.
- **pH-Insensitive**, *iFluor*™ dyes are robust and highly fluorescent over a broad pH range with little pH sensitivity.

Rapid Labeling of Proteins with RediLink™ Kits

RediLink™ *iFluor*™ protein labeling kits provide a convenient way to label proteins using the succinimidyl ester (SE) reactive form of the *iFluor*™ dyes. The succinimidyl ester shows good reactivity and selectivity with the aliphatic amines of proteins and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond. *iFluor*™-protein conjugates may be used for immunofluorescent staining, fluorescent *in situ* hybridization, flow cytometry and other biological applications.

Each RediLink™ *iFluor*™ protein labeling kit provides all the essential components for performing the conjugation reaction with a simple mixing step. The labeling kits require minimal hands-on time.

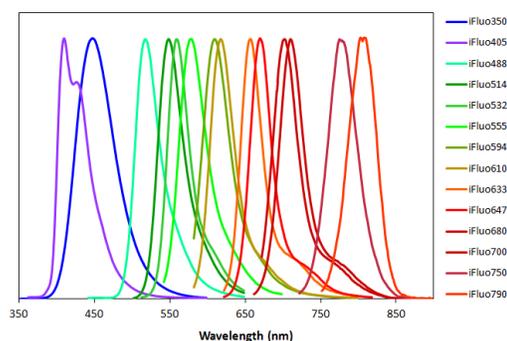


Figure 5.1. The emission spectra of *iFluor*™ Goat Anti-Rabbit IgG conjugates.

Table 5.1. Dye Selection Guide for iFluor™ Fluorescence-Labeling Dyes

iFluor™ Dye	Ex (nm)	Em (nm)	Features and Benefits	Ordering Information	Price
iFluor™ 350	345	442	Alternative to Alexa Fluor® 350 and DyLight™ 350 • Much stronger absorption • Much stronger fluorescence • Less environment-sensitive	Cat# 1060 (maleimide, SH-reactive)	\$95
				Cat# 1020 (SE, NH ₂ -reactive)	\$95
iFluor™ 405	401	420	Alternative to Cascade Blue®, Alexa Fluor® 405 and DyLight™ 405 • pH-insensitive fluorescence • Photostable	Cat# 1021 (SE, NH ₂ -reactive)	\$95
iFluor™ 488	491	514	Alternative to Alexa Fluor® 488 and DyLight™ 488 • pH-insensitive fluorescence • High labeling efficiency • Photostable	Cat# 1062 (maleimide, SH-reactive)	\$395
				Cat# 1023 (SE, NH ₂ -reactive)	\$295
iFluor™ 555	559	569	Alternative to Cy3®, Alexa Fluor® 555 and DyLight™ 550 • Strong fluorescence • More photostable than Cy3®	Cat# 1063 (maleimide, SH-reactive)	\$295
				Cat# 1028 (SE, NH ₂ -reactive)	\$195
iFluor™ 594	592	614	Alternative to Texas Red®, Alexa Fluor® 594 and DyLight™ 594 • Strong fluorescence • Photostable	Cat# 1029 (SE, NH ₂ -reactive)	\$195
iFluor™ 633	638	655	Alternative to Alexa Fluor® 633 • Strong fluorescence • Photostable	Cat# 1030 (SE, NH ₂ -reactive)	\$195
iFluor™ 647	654	674	Alternative to Cy5®, Alexa Fluor® 647 and DyLight™ 650 • Strong fluorescence • More photostable than Cy5®	Cat# 1065 (maleimide, SH-reactive)	\$295
				Cat# 1031 (SE, NH ₂ -reactive)	\$195
iFluor™ 680	682	701	Alternative to Cy5.5®, IRDye® 700, Alexa Fluor® 680 and DyLight™ 680 • Strong fluorescence • More photostable than Cy5.5®	Cat# 1066 (maleimide, SH-reactive)	\$295
				Cat# 1035 (SE, NH ₂ -reactive)	\$195
iFluor™ 700	693	713	Alternative to Alexa Fluor® 700 • Strong fluorescence • Good photostability	Cat# 1067 (maleimide, SH-reactive)	\$295
				Cat# 1036 (SE, NH ₂ -reactive)	\$195
iFluor™ 750	753	779	Alternative to Alexa Fluor® 750 and DyLight™ 750 • Stronger fluorescence • More photostable than Cy7®	Cat# 1068 (maleimide, SH-reactive)	\$295
				Cat# 1037 (SE, NH ₂ -reactive)	\$195
iFluor™ 790	782	811	Alternative to IRDye® 800, Alexa Fluor® 790 and DyLight™ 800 • Stronger fluorescence • Higher Photostability	Cat# 1066 (maleimide, SH-reactive)	\$295
				Cat# 1368 (SE, NH ₂ -reactive)	\$195

Table 5.2. Readilink™ iFluor™ Protein Labeling Kits

Cat. #	Product Description	Size	Price
1220	Readilink™ iFluor™ 350 Protein Labeling Kit	1 kit	\$195
1255	Readilink™ iFluor™ 488 Protein Labeling Kit	1 kit	\$195
1227	Readilink™ iFluor™ 555 Protein Labeling Kit	1 kit	\$195
1230	Readilink™ iFluor™ 594 Protein Labeling Kit	1 kit	\$195
1260	Readilink™ iFluor™ 633 Protein Labeling Kit	1 kit	\$195
1235	Readilink™ iFluor™ 647 Protein Labeling Kit	1 kit	\$195
1240	Readilink™ iFluor™ 680 Protein Labeling Kit	1 kit	\$195
1245	Readilink™ iFluor™ 700 Protein Labeling Kit	1 kit	\$195
1250	Readilink™ iFluor™ 750 Protein Labeling Kit	1 kit	\$195
1265	Readilink™ iFluor™ 790 Protein Labeling Kit	1 kit	\$195

Monitor Protease Contamination with Amplitude™ Fluorimetric Assay Kits

Protease assays are widely used for the investigation of protease inhibitors and detection of protease activities. Monitoring various protease activities has become a routine task for many biological laboratories. Some proteases have been identified as good drug development targets. On the other hand, many proteins are subject to protease-induced degradation, thus monitoring sample protease activities is often required in a variety of biological processes.

Our Amplitude™ Universal Fluorimetric Protease Activity Assay Kits are an ideal

choice to perform routine assays for the isolation of proteases, or for identifying the presence of contaminants in protein samples. The kits use a fluorescent casein conjugate which is proven to be a generic substrate for a broad spectrum of proteases (e.g. trypsin, chymotrypsin, thermolysin, proteinase K, protease XIV, and elastase). In the intact substrate, casein is heavily labeled with a fluorescent dye, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly fluorescent dye-labeled short peptides. The increase in fluorescence

intensity is directly proportional to protease activity.

The assay can be performed in a convenient 96-well or 384-well microtiter plate format and readily adapted to automation. For Kit 13500, the fluorescent signal can be easily read with a fluorescence microplate reader at Ex/Em = 490/525 nm using FITC filter set. For Kit 13501, the fluorescent signal can be easily read at Ex/Em = 540/590 nm. Both Kit 13500 and 13501 have been used for screening protease inhibitors in a HTS mode.

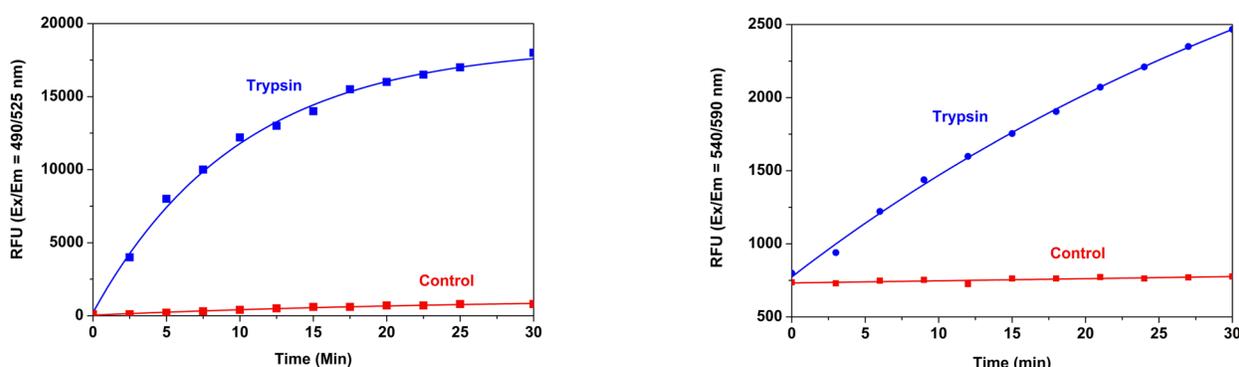


Figure 6.1. Trypsin protease activity was analyzed by Amplitude™ Universal Fluorimetric Protease Activity Assay kits (Cat. #13500 on the left, and Cat. #13501 on the right). Protease substrate was incubated with 1 unit trypsin for #13500 and 3 units for #13501. The control wells had protease substrate only (without trypsin). The fluorescence signal was measured starting from time 0 when trypsin was added. Samples were done in triplicate.

Key Features of Amplitude™ Protease Detection Kits:

- **Convenient format**, all the key assay components included.
- **Optimized performance**, optimal conditions for the detection of generic protease activity.
- **Continuous**, easily adapted to automation without a separation step.
- **Convenient**, formulated to have minimal hands-on time. No wash is required.
- **Non-radioactive**, no special requirements for waste treatment.

Table 6.1. Product Ordering Information

Cat. #	Product Description	Size	Price
13500	Amplitude™ Universal Fluorimetric Protease Activity Assay Kit *Green Fluorescence*	500 Assays	\$195
13501	Amplitude™ Universal Fluorimetric Protease Activity Assay Kit *Red Fluorescence*	500 Assays	\$195

Detect Thiol with Amplite™ Assay Kits

The detection and measurement of free thiol (such as free cysteine, glutathione, and cysteine residues in proteins) is one of the essential tasks for investigating biological processes and events in many biological systems. There are a few reagents or assay kits available for quantitating thiol content in biological systems. All the commercial kits either lack sensitivity or have tedious protocols.

Our Amplite™ Fluorimetric Thiol Quantitation Assay Kit provides an ultrasensitive fluorimetric assay to quantitate thiol that exists either in a small molecule or on a protein. The proprietary non-fluorescent dye used in the kit becomes strongly fluorescent upon reacting with thiol. The kit can detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume (10 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The thiol sensor used in the kit generates a strongly fluorescent adduct upon reacting with a thiol compound. The resulted adduct has the spectral properties almost identical to those of fluorescein. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making this assay kit highly robust. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

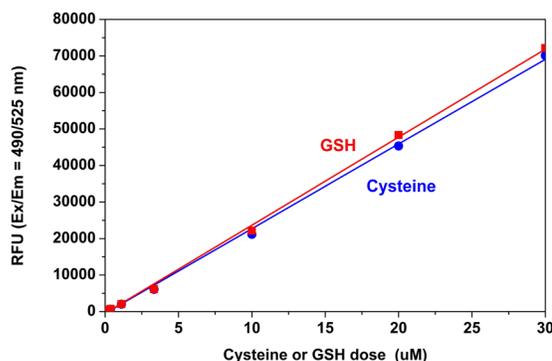


Figure 6.2. GSH and cysteine dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVostar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH or Cysteine was detected with 10 minutes incubation (n=3).

Detect Calcium by Absorbance or Fluorescence

Calcium is essential for all living organisms, particularly in cell physiology. Calcium also plays an important role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion.

Amplite™ Fluorimetric Calcium Quantitation Kit (Cat. #36360) provides a simple method for detecting calcium in physiological solutions by using our proprietary red fluorescence probe. The fluorescence signal can be easily read with a fluorescence microplate reader at Ex/Em = 540/590 nm. With the Amplite™ Fluorimetric Calcium Quantitation Kit, we have detected as little as 0.03 mM calcium. The kit has a broad dynamic range (30 µM to 10 mM). Amplite™ Colorimetric Calcium Quantitation Kit (Cat. #36361) uses our Calcium Blue™ as the chromogenic calcium indicator. Its absorbance changes in response to calcium binding. Calcium Blue™ binds calcium tightly in the neutral pH range, generating a Calcium Blue™-calcium complex that has intense absorption at ~650 nm.

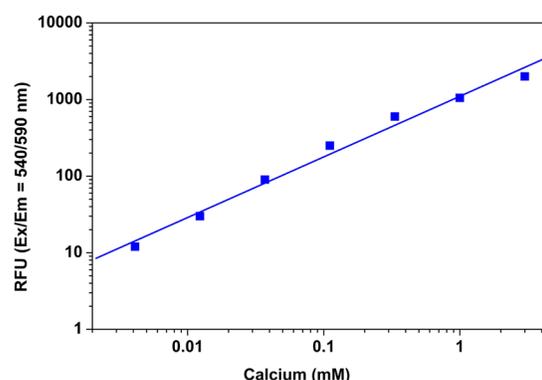


Figure 6.3. Calcium dose response was measured in a 96-well black solid plate with the Amplite™ Fluorimetric Calcium Quantitation Kit (Cat. # 36360). As low as 0.03 mM calcium was detected with 5 minutes incubation (n=3).

Table 6.2. Product Ordering Information

Cat. #	Product Description	Size	Price
36360	Amplite™ Fluorimetric Calcium Quantitation Kit *Red Fluorescence*	200 Assays	\$195
36361	Amplite™ Colorimetric Calcium Quantitation Kit *Blue Color*	200 Assays	\$195
5524	Amplite™ Fluorimetric Thiol Quantitation Assay Kit *Green Fluorescence*	200 Assays	\$195
10056	Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit *Green Fluorescence*	100 Assays	\$295
21507	Thiolite™ Blue	5 mg	\$95
21508	Thiolite™ Green	5 mg	\$145

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>

Beijing 4A Biotech Co., Ltd
Email: Info@4abio.com
Website: <http://www.4abio.com>

Beijing Zhonghao Shidai Co., Ltd
Email: info@biopcr.com
Website: <http://www.biopcr.com>

Tianjin Biolite Biotech Co., Ltd
Email: biolitebiotech@163.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: interchim@interchim.com
Website: <http://www.interchim.com>

Germany:

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

Hungary:

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

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>

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>

Interchim
Email: interchim@interchim.com
Website: <http://www.interchim.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>

Curiox Biosystems Pte Ltd.
Email: sales@curiox.com
Website: <http://www.curiox.com>

Slovakia:

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