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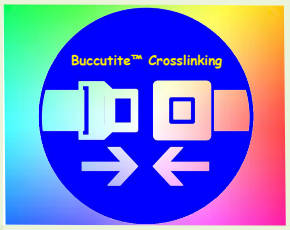

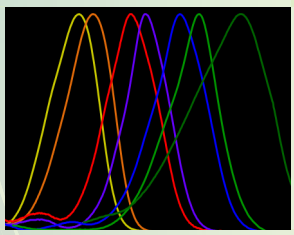
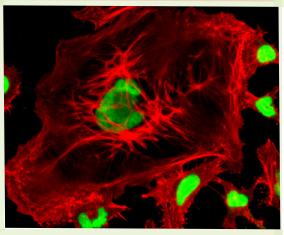

AAT Bioquest®
Advancing Assay & Test Technologies

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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 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

Maleimide Assays

Sensitive assays of maleimide and thiol groups are required for monitoring 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 introduced into the first protein.

Maleimides can be directly assayed spectrophotometrically at 302 nm. However, the small extinction coefficient of 620 $M^{-1}cm^{-1}$ renders the 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.

AAT Bioquest offers the most comprehensive solutions for detecting maleimide group. Table 1.1 summarizes the features and applications of our maleimide assay kits. These kits have been used for quantifying maleimide groups of different bioconjugates and other materials. Amplite™ Rapid Colorimetric Maleimide Quantitation Kit (Cat# 5526) has been specifically optimized for bioconjugates. We strongly recommend you use Kit 5526 for the rapid and accurate quantification of maleimide-containing proteins, oligos and nucleic acids.

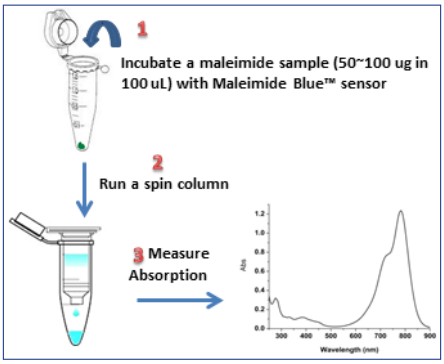

Figure 1.1. The overview of Amplite™ Rapid Colorimetric Maleimide Quantitation Kit (Cat# 5526).

Amplite™ Rapid Colorimetric Maleimide Quantitation Kit (Cat# 5526) uses our proprietary maleimide sensor Maleimide Blue™ with the maximum absorbance at ~780 nm. The principle of this assay is that Maleimide Blue™ reacts with a maleimide-containing sample, and the resulted product is run through a single spin column to remove the excess sensor. The absorption spectrum of the purified product is measured, and the maleimide to protein ratio can be calculated by the absorbance ratio of 780 nm/280 nm (for proteins) or 780 nm/260 nm (for oligos and nucleic acids). Amplite™ Rapid Colorimetric Maleimide Quantitation Kit can be performed using a traditional cuvette spectrophotometer, NanoDrop™ spectrophotometer, or a convenient 96-well absorbance plate reader with a UV-transparent plate.

Amplite™ Colorimetric Maleimide Quantitation Assay Kit (Cat# 5525) quantifies a maleimide group 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 $M^{-1}cm^{-1}$. 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 GSH thiol with the maleimide moiety. Maleimide in 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.

Amplite™ Fluorimetric Maleimide Quantitation Kit (Cat# 5523) 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. Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 490/520 nm. Compared to kit 5525, this fluorimetric assay is more sensitive, and has less interference from biological samples.

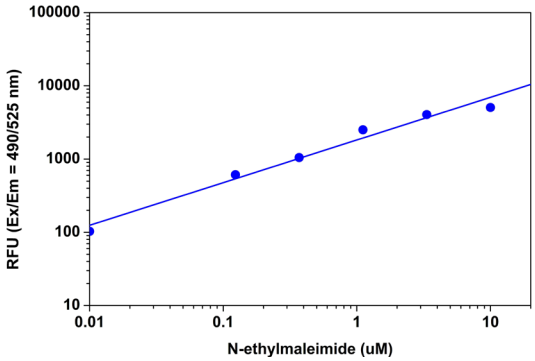

Figure 1.2. N-ethylmaleimide dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit (Cat# 5523).

Table 1.1 Assay Kits for Quantifying Maleimide Groups

Cat #	Product Name	Detection Mode	Feature	Recommended Use	Size	Ex (nm)	Em (nm)
5525	Amplite™ Colorimetric Maleimide Quantitation Kit	Absorption	Broad application	Nano particles and other materials	100 tests	324	N/A
5523	Amplite™ Fluorimetric Maleimide Quantitation Kit	Fluorescence	High sensitivity	Small molecules and soluble bioconjugates	200 tests	490	515
5526	Amplite™ Rapid Colorimetric Maleimide Quantitation Kit	Absorption	High accuracy	Proteins, oligos and nucleic acids	2 tests	780	N/A

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Glycerol Assays

Glycerol is a precursor for synthesis of triglycerides and phospholipids in liver and adipose tissue. When fasting, triglycerides stored in these lipid droplets can be hydrolyzed to generate free glycerol and fatty acids. The amount of free glycerol released to the bloodstream is proportional to the triglyceride/fatty acid cycling rate, which is important in the metabolic regulation and heat production.

Amplite™ Colorimetric Glycerol Assay Kit (Cat# 13832) offers a sensitive assay for measuring glycerol levels in biological samples. This assay is based on an enzyme coupled reaction of glycerol, in which the product hydrogen peroxide can be detected using Amplite™ Red substrate in the HRP-coupled reactions. The signal can be measured with an absorbance microplate reader using OD ratio of 570 nm/610 nm. With Amplite™ Colorimetric Glycerol Assay Kit, as low as 0.15 µg/mL (~1.6 µM) glycerol was detected in a 100 µL reaction volume.

Amplite™ Fluorimetric Glycerol Assay Kit (Cat# 13833) is also based on an enzyme coupled reaction of glycerol, in which the product hydrogen peroxide can be detected using Amplite™ Red substrate in HRP-coupled reactions. The fluorescence signal can be measured using a fluorescence microplate reader at Ex/Em= 540/590 nm. With Amplite™ Fluorimetric Glycerol Assay Kit, as low as 0.015 µg/mL (~0.16 µM) glycerol was detected in a 100 µL reaction volume.

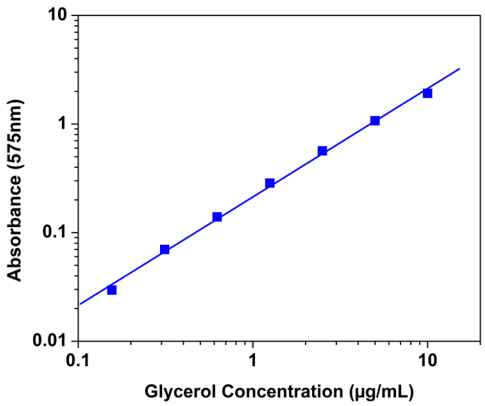

Figure 1.3. Glycerol dose responses were measured with Amplite Colorimetric Glycerol Assay Kit (Cat# 13832) on a 96-well black wall/clear bottom plate.

Glycerol 3-Phosphate Assays

Glycerol 3-phosphate is an important intermediate in the glycolysis metabolic pathway. Animals, fungi, and plants use glycerol 3-Phosphate to produce ATP. It is used to regenerate NAD+ in brain and skeletal muscle cells. Glycerol 3-phosphate has been linked to lipid imbalance diseases such as obesity.

Amplite™ Glycerol 3-Phosphate Assay Kits (Cat# 13837 and 13838) provide one of the most sensitive methods for quantifying glycerol 3-phosphate. The kits use Amplite™ Red substrate to quantify the concentration of glycerol 3-phosphate, which is related to the production of hydrogen peroxide in the glycerol 3-phosphate oxidase-mediated enzyme coupling reactions. The amount of glycerol 3-phosphate is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. The kits are an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

Amplite™ Colorimetric Glycerol 3-Phosphate Assay Kit (Cat# 13838) detects as little as 12.5 µM glycerol 3-phosphate in 100 µL assay volume. 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 with an absorbance microplate reader at ~576±5 nm.

Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat# 13837) detects as little as 41 picomole glycerol 3-phosphate in 100 µL assay volume (0.41 µM). 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 with a fluorescence microplate reader at Ex/Em = ~540/590 nm.

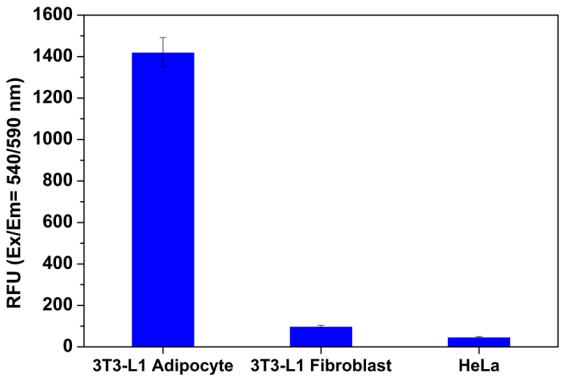

Figure 1.4. Measurements of glycerol 3-phosphate using Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat# 13837) in 3T3-L1 adipocyte, 3T3-L1 fibroblast and HeLa cell lysates. Cells (1×10^5) were lysed using ReadiUse™ Mammalian Cell Lysis Buffer (Cat# 20012), and then 50 µL of cell lysate was used as glycerol 3-phosphate containing test samples. Assay was performed following the kit protocol.

Table 1.2 Glycerol & Glycerol 3-Phosphate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13832	Amplite™ Colorimetric Glycerol Assay Kit	200 tests	575	N/A
13838	Amplite™ Colorimetric Glycerol 3-Phosphate Assay Kit *Red Color*	200 tests	575	N/A
13833	Amplite™ Fluorimetric Glycerol Assay Kit	200 tests	571	585
13837	Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit *Red Fluorescence*	200 tests	571	585

Acetylcholinesterase Assays

Acetylcholinesterase (AChE) is one of the most crucial enzymes for nerve response and function. AChE degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate the synaptic transmission. AChE inhibitors are among the key drugs approved for Alzheimer's disease (AD) and myasthenia gravis.

Amplite™ Fluorimetric Acetylcholinesterase Assay Kits provide one of the most sensitive methods for detecting AChE activity or screening AChE inhibitors in red florescence window. Kit 11402 uses Amplite™ Red to quantify the choline produced from the hydrolysis of acetylcholine by AChE through choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite™ Red is used to measure the amount of choline formed, which is proportional to the AChE activity. Kit 11402 can be used for monitoring and quantifying the AChE activity in blood, cell extracts or other solutions. The kit is an optimized "mix and read" assay that provides a simple one-step fluorimetric assay to detect as little as 0.01 mU AChE in a 100 µL assay volume (0.1 mU/mL). Its signal can be easily read at Ex/Em = ~540/590 nm.

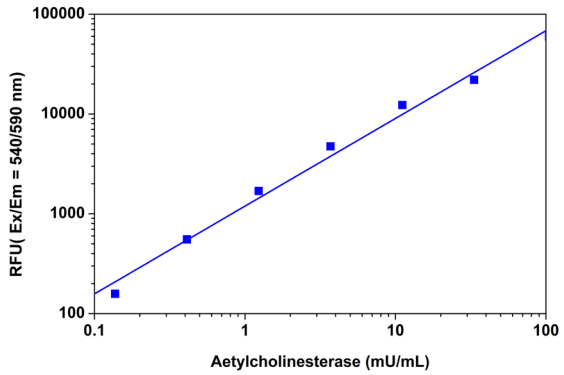

Figure 1.5. Acetylcholinesterase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit (Cat# 11402).

Amplite™ Fluorimetric Acetylcholinesterase Assay Kit (Cat# 11401) uses our outstanding Thiolite™ Green to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE. Thiolite™ Green is not fluorescent until reacted with a thiol group. It has spectral properties similar to those of fluorescein, making this assay compatible with almost all fluorescence instruments. The fluorescence intensity of Thiolite™ Green is used to measure AChE activity. Compared to the existing thiol probes (e.g., mBBr and bBBr), Thiolite™ Green is much more sensitive. This Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides an ultrasensitive fluorimetric one-step assay to detect as little as 0.01mU AChE in a 100 µL assay volume (0.1 mU/mL). Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 490/525 nm.

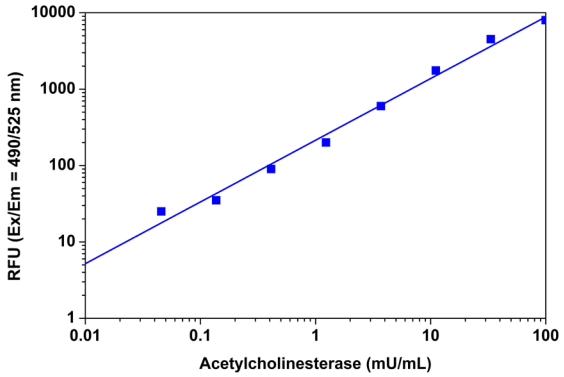

Figure 1.6. Acetylcholinesterase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit (Cat# 11401).

Amplite™ Colorimetric Acetylcholinesterase Assay Kit (Cat# 11400) uses DTNB to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in blood, in cell extracts, and in other solutions. The absorption intensity of DTNB adduct is used to measure the amount of thiocholine formed, which is proportional to the AChE activity.

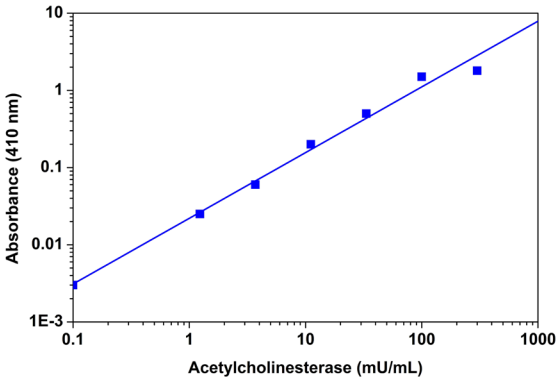

Figure 1.7. Acetylcholinesterase dose responses were measured in a 96-well clear plate with Amplite™ Colorimetric Acetylcholinesterase Assay Kit (Cat# 11400).

Table 1.3 Acetylcholinesterase Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
40007	Amplite™ Choline Quantitation Kit	200 tests	571	585
11400	Amplite™ Colorimetric Acetylcholinesterase Assay Kit	200 tests	410	N/A
11403	Amplite™ Fluorimetric Acetylcholine Assay Kit *Red Fluorescence*	200 tests	571	585
11401	Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Green Fluorescence*	200 tests	510	524
11402	Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Red Fluorescence*	200 tests	571	585

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Nucleic Acid Quantification

dsDNA Assays

Helixyte™ Green Fluorimetric dsDNA Assay Kits (Cat# 17650 & 17651) simplify DNA quantification without sacrificing sensitivity. The assay provides a linear detection range between 0.2 ng and 1000 ng double-stranded DNA (dsDNA) (Figure 1.8). The high-sensitivity DNA assay is ideal for quantifying PCR products, viral DNA, DNA fragments for subcloning and other applications requiring small amounts of DNA. Helixyte™ Green Fluorimetric dsDNA Assay Kits are highly selective for dsDNA over RNA and other common contaminants, including free nucleotides, salts, solvents and proteins. The Helixyte™ Green assay is an excellent replacement for PicoGreen®-based DNA assays.

Helixyte™ Green dsDNA Quantifying Reagent (Cat# 17597) can accurately quantify as little as 100 pg/mL of dsDNA using a fluorometer or 300 pg/mL using a fluorescence microplate reader. Helixyte™ Green dsDNA quantification assay is 10,000 times more sensitive than conventional UV absorbance measurements at 260 nm and at least 400 times more sensitive than the Hoechst 33258 dye–based assay. Helixyte™ Green dsDNA Quantifying Reagent shows >1000-fold fluorescence enhancement upon binding to dsDNA, and much less fluorescence enhancement upon binding to single-stranded DNA (ssDNA) or RNA, making it possible to quantify dsDNA in the presence of equimolar amounts of ssDNA, RNA or proteins. Helixyte™ Green dsDNA Quantifying Reagent shows little if any AT- or GC-selectivity, enabling accurate DNA quantification.

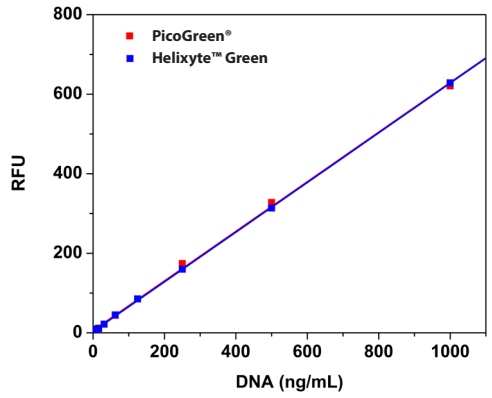

Figure 1.8. The quantification of calf thymus DNA with Helixyte™ Green vs. PicoGreen®.

RNA Assays

StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17610) provides a homogeneous assay for quantifying RNA in the presence of DNA. This RNA assay exhibits a linear detection range between 5 ng and 100 ng RNA (Figure 1.9). Assay linearity is maintained even in the presence of several interfering compounds commonly found in nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins and agarose. Its relatively high selectivity for RNA over dsDNA enables accurate RNA quantification in the presence of DNA and other common contaminants, including free nucleotides, salts, solvents and proteins, making this assay ideal for measuring samples for microarray, RT-PCR and northern blot procedures.

StrandBrite™ Green Fluorimetric RNA Quantitation Kit allows detection of as little as 10 ng/mL RNA with a standard fluorometer, fluorescence microplate reader or filter-based fluorometer using standard fluorescein excitation and emission settings. The sensitivity is at least 20-fold better than that achieved with ethidium bromide and at least 100-fold better than that achieved using conventional absorbance measurements at 260 nm. Unlike UV absorbance measurements at 260 nm, StrandBrite™ Green RNA Quantifying Reagent (Cat# 17611) does not detect significant sample contamination caused by free nucleotides. Thus, StrandBrite™ Green RNA Quantifying Reagent more accurately measures the amount of intact RNA polymers in potentially degraded samples.

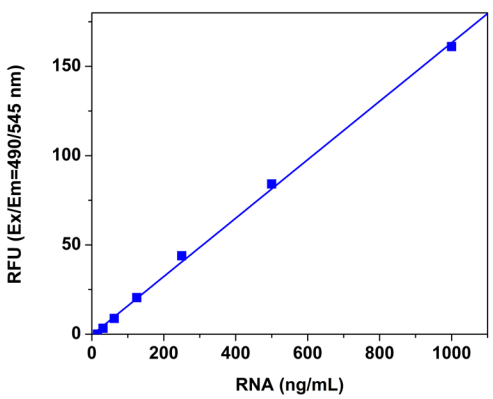

Figure 1.9. The quantification of RNA with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17610).

Table 1.4 Fluorescent Probes for Quantifying DNA & RNA Samples in Solution

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17597	Helixyte™ Green dsDNA Quantifying Reagent	1 mL	501	520
17651	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit	200 tests	501	520
17650	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *Optimized for Microplate Reader*	200 tests	501	520
17610	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *Optimized for Microplate Readers*	200 tests	501	520
17611	StrandBrite™ Green RNA Quantifying Reagent	1 mL	501	520

Multicolor Intracellular Calcium Detection Probes

Cal-520™ Calcium Detection Probes

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). Cal-520™ AM provides the most 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. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increases the fluorescence of Cal-520™ AM. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement, make Cal-520™ AM an ideal indicator for the measurement of intracellular calcium. The high signal to background ratio and better intracellular retention make the Cal-520™ AM calcium assay an ideal tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

AAT Bioquest offers a full range of Cal-520™ calcium detection probes. Cal-520FF™ is used for monitoring calcium in high concentration. The dextran forms of our calcium indicators show a dramatic reduction in both leakage and compartmentalization compared to the AM ester forms. Among the fluorescent calcium indicator dextran conjugates, Cal-520™-dextran conjugates might be the best choice due to their high fluorescence quantum yields and large fluorescence enhancements by calcium. Compared to Oregon Green® BAPTA-1 dextran, Cal-520™-dextran exhibits much lower background and much larger calcium-induced fluorescence enhancement. Cal-520™-biotin and Cal-520™-biocytin conjugates have great calcium responses. Their avidin and streptavidin complexes have sensitive calcium responses as well. Conceivably Cal-520™-biotin and Cal-520™-biocytin conjugates could be bound to an IgG-avidin or an IgG-streptavidin conjugate for monitoring calcium change spatially for a specific target. Our amine-reactive Cal-520™ NHS ester and thiol-reactive Cal-520™ maleimide could be coupled to an IgG or other carrier molecules to prepare a calcium-sensitive bioconjugates for monitoring calcium change spatially for a specific target.

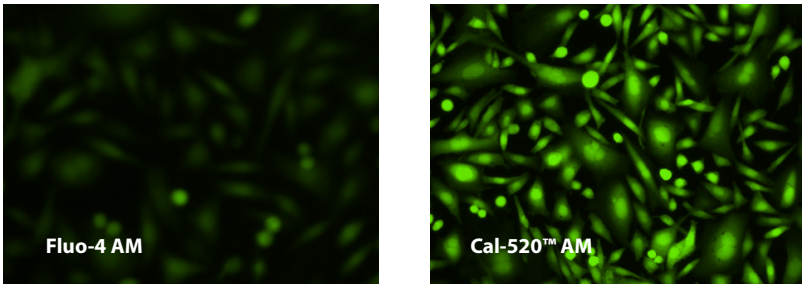

Figure 2.1. Responses of endogenous P2Y receptor to ATP in CHO-M1 cells without probenecid. CHO-M1 cells were seeded overnight at 40,000 cells per 100 µL per well in a Costar 96-well black wall/clear bottom plate. 100 µL of 4 µM Fluo-4 AM (left), Cal-520™ AM (right) in HHBS was added into the wells,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

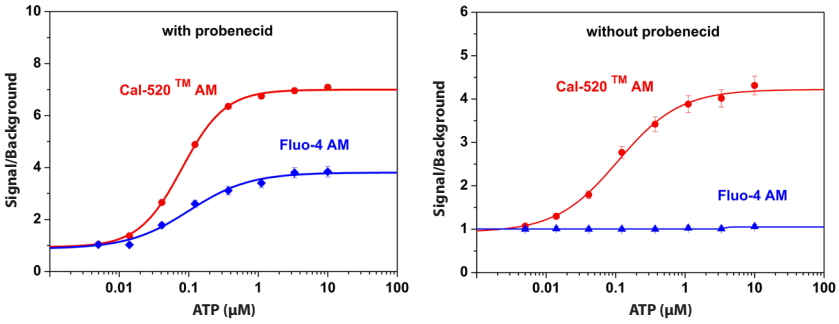

Figure 2.2. ATP-stimulated calcium responses of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-520™ AM (red curve), or Fluo-4 AM (blue curve) respectively with (left) or without probenecid (right) under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100 µL per well in a Costar 96-well black wall/clear bottom 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 2.1 Spectral Comparison of Fluo-3, Fluo-4, Fluo-8® and Cal-520™

Dye	Ex (nm)	Em (nm)	QY*	K_d (nM)
Cal-520™	492	514	**0.75**	320
Fluo-3	506	525	0.15	390
Fluo-4	493	515	0.16	345
Fluo-8®	490	514	0.16	389

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

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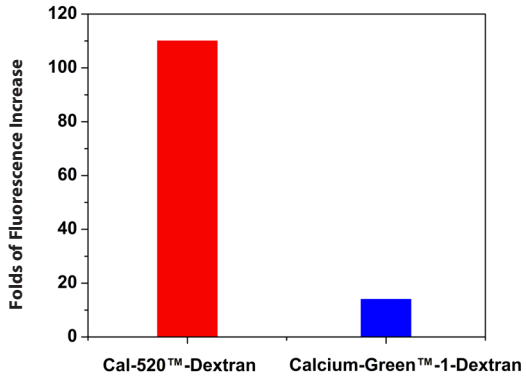

Figure 2.3. Calcium response comparison of Cal-520™-dextran with Calcium Green™ -1-dextran in the presence of saturated calcium citrate.

Cal-590™ Calcium Detection Probes

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Rhod-2 AM is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses.

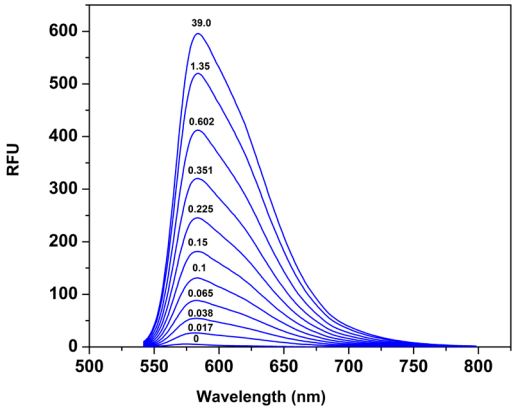

Figure 2.4. Fluorescence emission spectra of Cal-590™ in solutions containing 0 to 39 μM free Ca^{2+}.

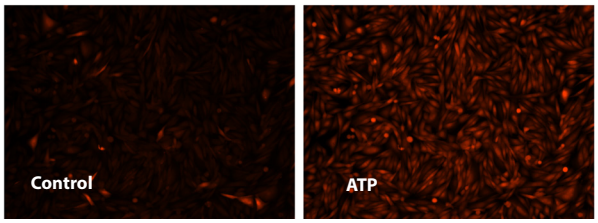

Figure 2.5. Responses of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 40,000 cells per 100 μL per well in a Costar 96-well black wall/clear bottom plate. 100 μL of 4 μM Cal-590™ AM (Cat# 20510) in HHBS with 1 mM probenecid were added into the wells, and the cells were incubated at 37 °C for 2 hours. The dye loading mediums were replaced with 100 μL HHBS and 1 mM probenecid , then imaged with a fluorescence microscope (Olympus IX71) using TRITC channel before and after adding 50 μL of 300 μM ATP .

Cal-590™ has been developed to improve Rhod-2 AM cell loading and calcium response while maintaining the similar spectral wavelengths of Rhod-2 AM, making it compatible with TRITC/Cy3® filter set. In CHO and HEK cells, the cellular calcium response of Cal-590™ is much more sensitive than that of Rhod-2 AM. The spectra of Cal-590™ is well separated from those of FITC, Alexa Fluor® 488 and GFP, making it an ideal calcium probe for multiplexing intracellular assays with GFP cell lines or FITC/Alexa Fluor® 488 labeled antibodies.

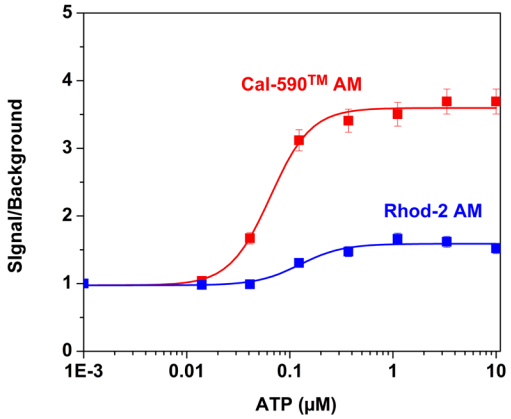

Figure 2.6. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-590™ AM (Red) and Rhod-2, AM (Blue) under the same conditions. CHO-K1 cells were seeded overnight at the cell density of 50,000 cells per 100 μL per well in a 96-well black wall/clear bottom plate. 100 μL of 5 μg/mL Cal-590™ AM or Rhod-2 AM with 2.5 mM probenecid was added into the cells, and the cells were incubated at 37 °C for 1 hour. ATP (50 μL/well) was added by FlexStation® (Molecular Devices) to achieve the final indicated concentrations.

Table 2.2 Cal-520™ and Cal-590™ Calcium Detection Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
21130	Cal-520™, AM	10x50 μg	492	514
20605	Cal-520™-Biotin Conjugate	5x50 μg	492	514
20606	Cal-520™-Biocytin Conjugate	5x50 μg	492	514
20600	Cal-520-Dextran Conjugate *MW 3,000*	1 mg	492	514
20601	Cal-520-Dextran Conjugate *MW 10,000*	5 mg	492	514
20610	Cal-520™ Maleimide	100 μg	492	514
20609	Cal-520™ NHS Ester	100 μg	492	514
21140	Cal-520™, Potassium Salt	10x50 μg	492	514
21135	Cal-520™, Sodium Salt	10x50 μg	492	514
21142	Cal-520FF™, AM	1 mg	492	514
21143	Cal-520FF™, AM	10x50 μg	492	514
21144	Cal-520FF™, Potassium Salt	10x50 μg	492	514
20510	Cal-590™, AM	5x50 μg	573	588
20511	Cal-590™, AM	10x50 μg	573	588
20512	Cal-590™, AM	1 mg	573	588
20518	Cal-590™, Potassium Salt	5x50 μg	573	588
20515	Cal-590™, Sodium Salt	5x50 μg	573	588

Cal-630™ Calcium Detection Probes

X-Rhod-1 is commonly used as a red fluorescent calcium indicator. However, X-Rhod-1 is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. In addition, X-Rhod-1 is mostly localized in mitochondria, thus giving low signal/background ratio. Cal-630™ has been developed to improve X-Rhod-1 cell loading and calcium response while maintaining the similar spectral wavelengths of X-Rhod-1, making it compatible with Texas Red® filter set. In CHO and HEK cells, the cellular calcium response of Cal-630™ is much more sensitive than that of X-Rhod-1. The maximum emission wavelength of Cal-630™ is well separated from those of FITC, Alexa Fluor® 488 and GFP, making it an ideal calcium probe for multiplexing intracellular assays with GFP cell lines or FITC/Alexa Fluor® 488 labeled antibodies.

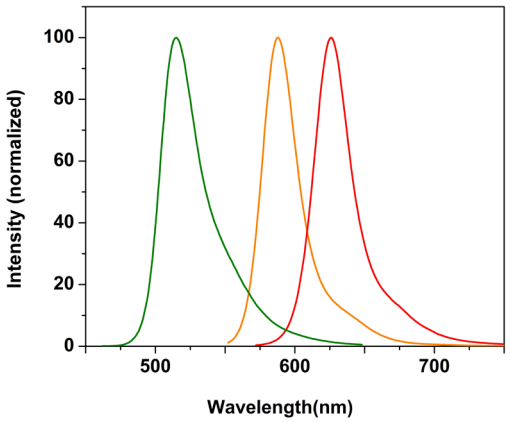

Figure 2.7. Normalized emission spectra of Cal-520™ (Green), Cal-590™ (Orange) and Cal-630™ (Red).

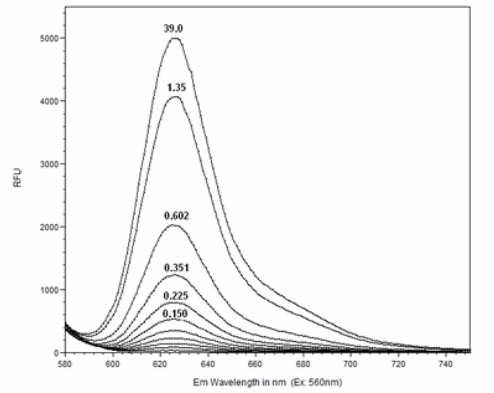

Figure 2.8. Fluorescence emission spectra of Cal-630™ in solutions containing 0 to 39 μM free Ca^{2+}.

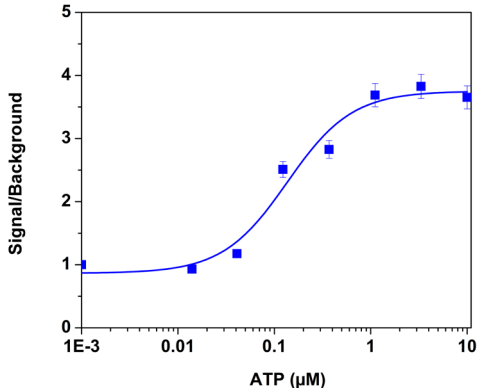

Figure 2.9. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells measured with Cal-630™ AM (Cat# 20530). CHO-K1 cells were seeded overnight at the cell density of 50,000 cells per 100 μL per well in a 96-well black wall/clear bottom plate. 100 μL of 10 μg/mL Cal-630™ AM with 2.0 mM probenecid was added into the cells, and the cells were incubated at 37 °C for 2 hours. ATP (50 μL/well) was added by FlexStation® (Molecular Devices) to achieve the final indicated concentrations.

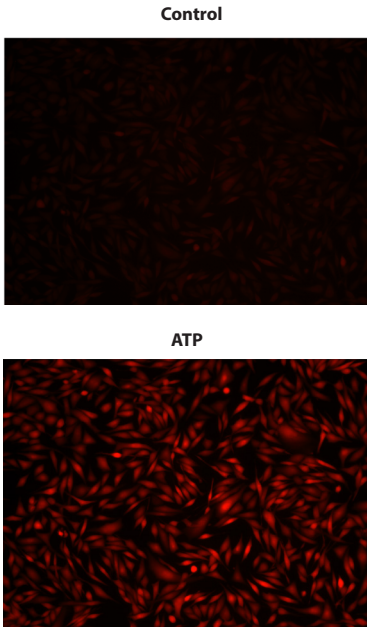

Figure 2.10. Responses of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 40,000 cells per 100 μL per well in a 96-well black wall/clear bottom plate. 100 μL of 4 μM Cal-630™ AM (Cat# 20530) in HHBS with 1 mM probenecid were added into the wells, and the cells were incubated at 37 °C for 2 hours. The dye loading mediums were replaced with 100 μL HHBS and 1 mM probenecid , then imaged with a fluorescence microscope (Olympus IX71) using TRITC channel before and after adding 50 μL of 300 μM ATP .

Table 2.3 Cal-630™ Calcium Detection Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)	K_d (nM)
20530	Cal-630™, AM	5x50 μg	608	626	792
20531	Cal-630™, AM	10x50 μg	608	626	792
20532	Cal-630™, AM	1 mg	608	626	792
20538	Cal-630™, Potassium Salt	5x50 μg	608	626	792
20535	Cal-630™, Sodium Salt	5x50 μg	608	626	792

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Intracellular Nitric Oxide (NO) Assays

Altered NO production is implicated in various immunological, cardiovascular, neurodegenerative and inflammatory diseases. As a free radical, NO is rapidly oxidized and exists in relatively low concentration. It has been challenging to detect and understand the role of NO in biological systems. Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kits provide a robust tool to monitor intracellular NO level in live cells.

Nitrixyte™ Orange and Nitrixyte™ Red are developed as excellent replacements for DAF-2 for the detection and imaging of free NO in cells. Compared to the widely used DAF-2 probes, Nitrixyte™ Orange and Nitrixyte™ Red have better photostability and enhanced cell permeability. Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kits (Cat# 16350 & 16351) use Nitrixyte™ Orange that reacts with NO to generate a bright orange fluorescent product. The NO-generated product of Nitrixyte™ Orange has spectral properties similar to those of Cy3® and TRITC. Nitrixyte™ Orange can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy3® or TRITC. Kit 16350 is optimized for fluorescence imaging and microplate reader applications. Kit 16351 is optimized for flow cytometry applications.

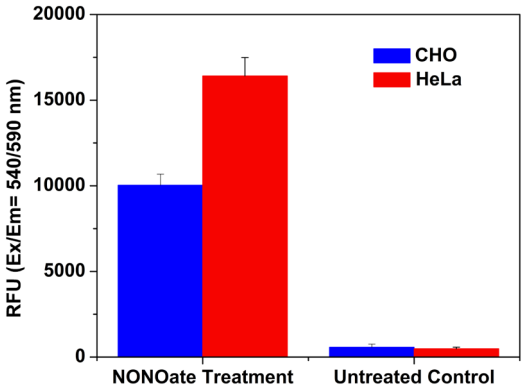

Figure 2.11. Detection of exogenous nitric oxide (NO) in cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16350). CHO-K1 and HeLa cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were incubated with Nitrixyte™ Orange working solution at 37 °C for 30 minutes. The cells were treated with or without 1mM DEA NONOate at 37 °C for 30 minutes. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode.

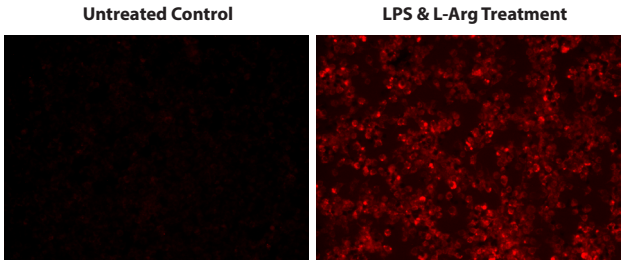

Figure 2.12. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16350). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were incubated with Nitrixyte™ Orange, and treated with (Right) or without (Left) 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) at 37 °C for 16 hours.

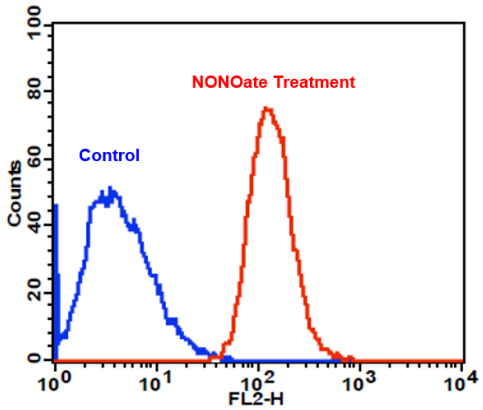

Figure 2.13. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16351). Cells were incubated with Nitrixyte™ Orange at 37 °C for 30 minutes and washed with assay buffer twice. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 30 minutes.

Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit 16356 uses Nitrixyte™ Red that reacts with NO to generate a bright red fluorescent product. The NO-generated fluorescent product of Nitrixyte™ Red has spectral properties similar to those of Texas Red®. Nitrixyte™ Red can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Texas Red®. This kit is optimized for flow cytometry applications.

Table 2.4 Intracellular Nitric Oxide (NO) Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
16351	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	100 tests	545	576
16350	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Orange Fluorescence Optimized for Microplate Reader*	200 tests	545	576
16356	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests	588	610

Intracellular Total ROS Activity Assays

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen (such as superoxide, hydroxyl radical, singlet oxygen and peroxides). ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and plays important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. It may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS is also generated by exogenous sources such as ionizing radiation. Under the conditions of oxidative stress, greatly increased production of ROS results in subsequent alteration of membrane lipids, proteins and nucleic acids. Oxidative damage of these biomolecules is associated with aging as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and neurodegenerative disorders.

Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kits provide a sensitive, one-step fluorimetric assay to detect intracellular ROS in live cells. 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 using either a fluorescence microplate reader or a fluorescence microscope. Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kits (Cat# 22900, 22901, 22902 and 22903) are in an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. Kit 22904 is optimized for flow cytometry applications, its signal can be detected at Ex/Em = 490/520 nm (FL1 channel).

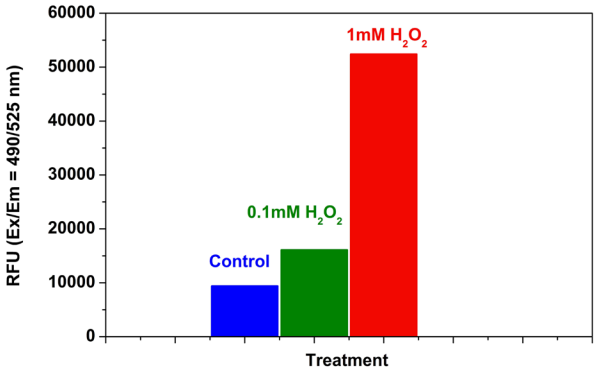

Figure 2.14. Detection of ROS in Jurkat cells using Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22900). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 ºC incubator for 1 hour. And then the cells were treated with 1 mM, 0.1 mM or without H_2O_2 for 30 minutes.

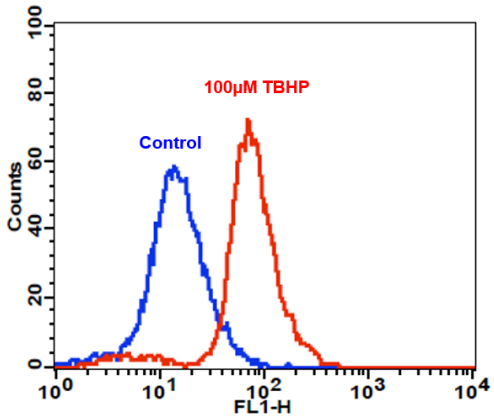

Figure 2.15. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22904). Cells were incubated with Amplite™ ROS Green at 37 ºC for 1 hour. Cells were then treated with (Red) or without (Blue) 100 µM TBHP at 37 ºC for 30 minutes. The fluorescence signal was monitored using a flow cytometer (BD FACSCalibur™) in FL1 channel.

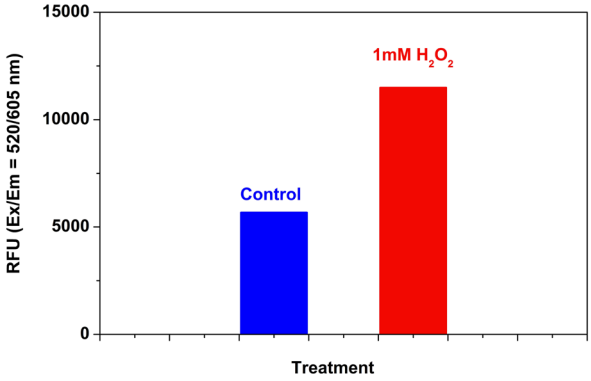

Figure 2.16. Detection of ROS in Jurkat cells using Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22901). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. And then the cells were treated with or without 1mM H_2O_2 for 2 hours.

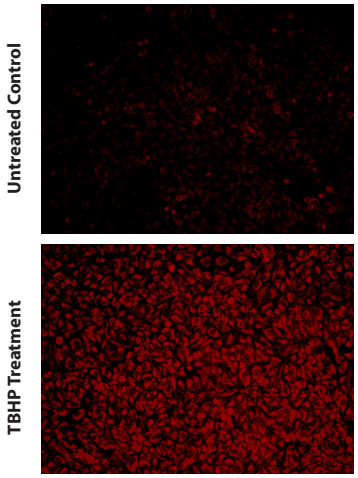

Figure 2.17. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22902) in a Costar 96-well black wall/clear bottom plate. Top: Untreated control cells. Bottom: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

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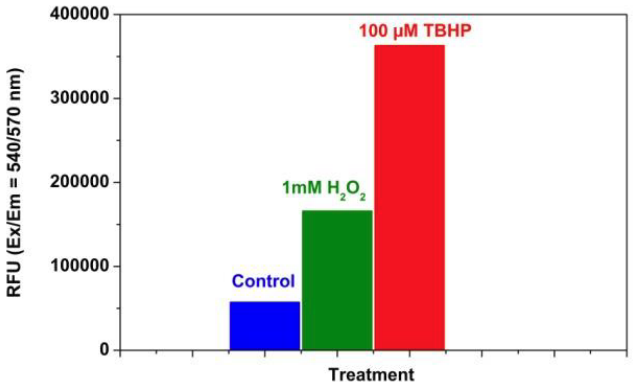

Figure 2.18. Detection of ROS in HeLa cells. HeLa cells were seeded overnight at 15,000 cells/90 µL/well in a Costar 96-well black wall/clear bottom plate. The cells were untreated (control) or treated with 1 mM H_2O_2 or 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. The ROS Brite™ 570 (Cat# 16000) assay solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence signal was monitored at Ex/Em = 540/570 nm (cut off = 550 nm) with bottom read mode using FlexStation® (Molecular Devices).

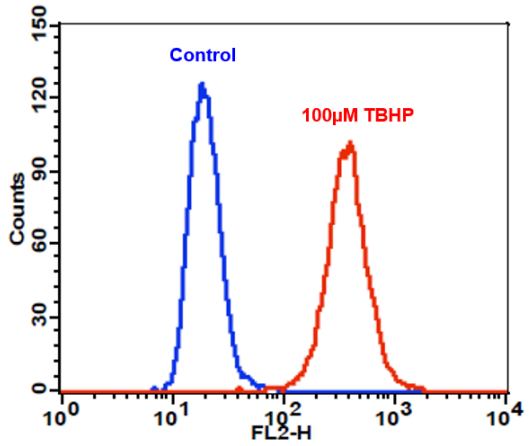

Figure 2.19. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 570 (Cat# 16000) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

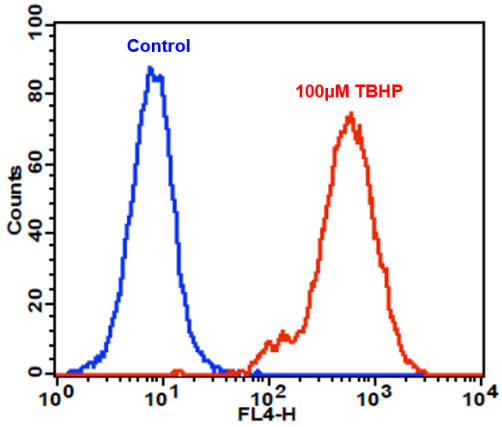

Figure 2.20. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 670 (Cat# 16002) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

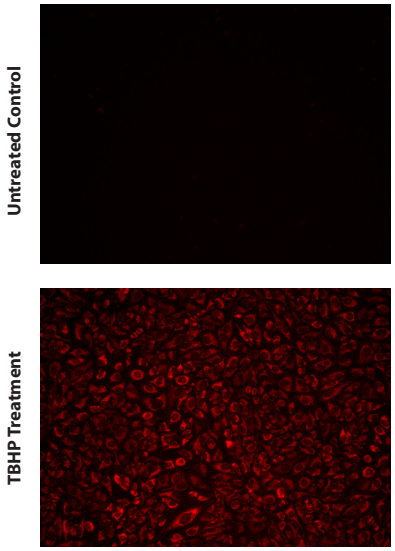

Figure 2.21. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22903) in a Costar 96-well black wall/clear bottom plate. Top: Untreated control cells. Bottom: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

Table 2.5 Intracellular Total ROS Activity Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22903	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Deep Red Fluorescence*	200 tests	639	660
22900	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Green Fluorescence*	200 tests	492	520
22904	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	492	520
22902	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Orange Fluorescence*	200 tests	556	566
22901	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Red Fluorescence*	200 tests	520	605

iFluor™ 700 Dyes

Spectrally similar to Alexa Fluor® 700 dyes, iFluor™ 700 dyes have fluorescence emission maximum at 710 nm with fluorescence quantum yields close to 0.2. Compared to Alexa Fluor® 700 dyes, iFluor™ 700 dyes are brighter with stronger absorption at 633 nm. Fluorescence emission of iFluor™ 700 dyes is well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin and iFluor™ 647 dyes, making it ideal for three or four-color labelings. iFluor™ 700 dyes can be effectively excited by the 633 nm red laser in flow cytometers, giving additional near infrared emission. iFluor™ 700 dyes are also excellent acceptor dyes for allophycocyanin (APC), further facilitating multicolor flow cytometry analysis. Our in-house research indicated that the iFluor™ 700-labeled antibodies generally give much higher stain index than the corresponding antibodies labeled with Alexa Fluor® 700 (See Figure 3.2).

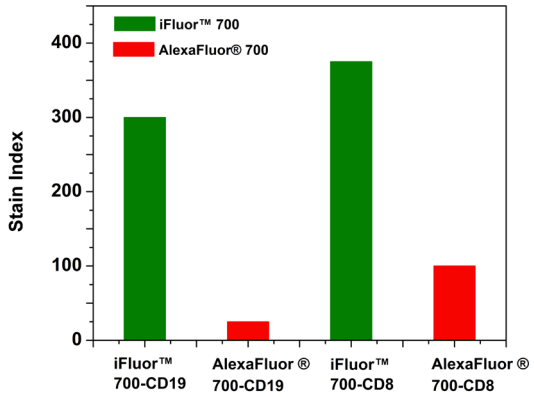

Figure 3.2. Stain index comparison of iFluor™ 700 with Alexa Fluor® 700.

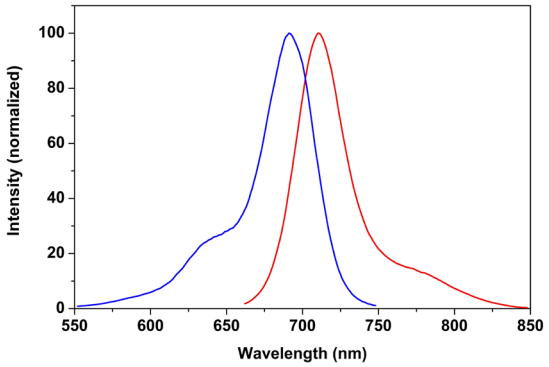

Figure 3.1. The excitation and emission spectra of iFluor™ 700 Goat Anti-Rabbit IgG Conjugate (Cat# 16652) in PBS buffer (pH 7.2).

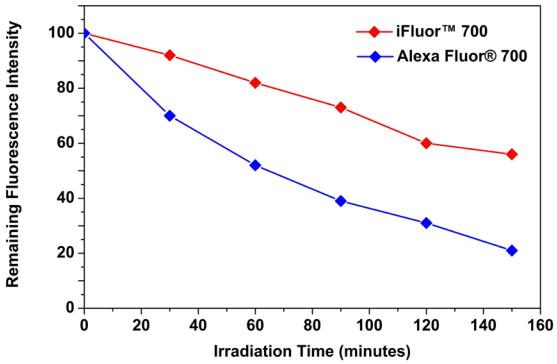

Figure 3.3. Photostability comparison of APC-iFluor™ 700 Tandem (Cat# 2570) with the spectrally equivalent APC-Alexa Fluor® 700 tandem in PBS buffer (pH 7.2). Both iFluor™ 700 and Alexa Fluor® tandems were irradiated with 200 W lamp in PBS (pH 7.2).

Table 3.1 iFluor™ 700 Dyes

Cat #	Product Name	Alternative to	Size	Ex (nm)	Em (nm)
20077	Annexin V-iFluor™ 700 Conjugate	Alexa Fluor® 700	100 tests	690	716
2570	APC-iFluor™ 700 Tandem	Alexa Fluor® 700	1 mg	651	713
16494	iFluor™ 700 Goat Anti-Mouse IgG (H+L)	Alexa Fluor® 700	200 µg	693	713
16574	iFluor™ 700 Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 700	200 µg	693	713
16652	iFluor™ 700 Goat Anti-Rabbit IgG (H+L)	Alexa Fluor® 700	200 µg	693	713
16714	iFluor™ 700 Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 700	200 µg	693	713
1087	iFluor™ 700 Hydrazide	Alexa Fluor® 700	1 mg	685	710
1067	iFluor™ 700 Maleimide	Alexa Fluor® 700	1 mg	685	710
16970	iFluor™ 700-Streptavidin Conjugate	Alexa Fluor® 700	200 µg	693	713
1036	iFluor™ 700 Succinimidyl Ester	Alexa Fluor® 700	1 mg	685	710
1245	ReadiLink™ iFluor™ 700 Protein Labeling Kit	Alexa Fluor® 700	2 labelings	693	713

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Buccutite™ Protein Crosslinking Technology

Protein-protein conjugations are commonly performed with a bifunctional linker (such as the commonly used SMCC), having different reactivity on each end for linking two different proteins. One end of the crosslinker reacts (via NHS ester) with amines (-NH2) found in the amino acid lysine and N-terminus, and the other end reacts (via maleimide) with the thiol groups (-SH) found in the amino acid cysteine. However, SMCC-modified protein is extremely unstable and often self-reactive since proteins often contain both amine and thiol groups that cause significant amount of homo-crosslinking. In addition, it is quite difficult and tedious to quantify the number of maleimide groups on a protein.

Buccutite™ crosslinking technology provides a new robust method to label proteins with another macromolecule (such as proteins, nucleic acids, oligos, peptides or other biopolymers). Buccutite™ crosslinking technology uses two small linking molecules that readily react with proteins through their amino groups (N-terminal or lysine residue), and the two resulted Buccutite™ pairs of macromolecules can be readily cross-linked by a simple mixing as shown in Figure 3.4.

Based on this new Buccutite™ crosslinking technology, ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit (Cat# 5503) has proven to be one of the most robust HRP labeling kits. The kit is designed for preparing horseradish peroxidase (HRP) conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The HRP provided has been pre-activated with our proprietary linker Buccutite™ FOL, and can be directly used for conjugation. The Buccutite™ FOL -activated HRP readily reacts with Buccutite™ MTA-containing molecules under extremely mild neutral conditions without any catalyst required. Compared to commonly used SMCC and other similar technologies, our Buccutite™ bioconjugation system is much more robust and easier to use. It enables faster and effective conjugation of biomolecules with higher efficiencies and yields.

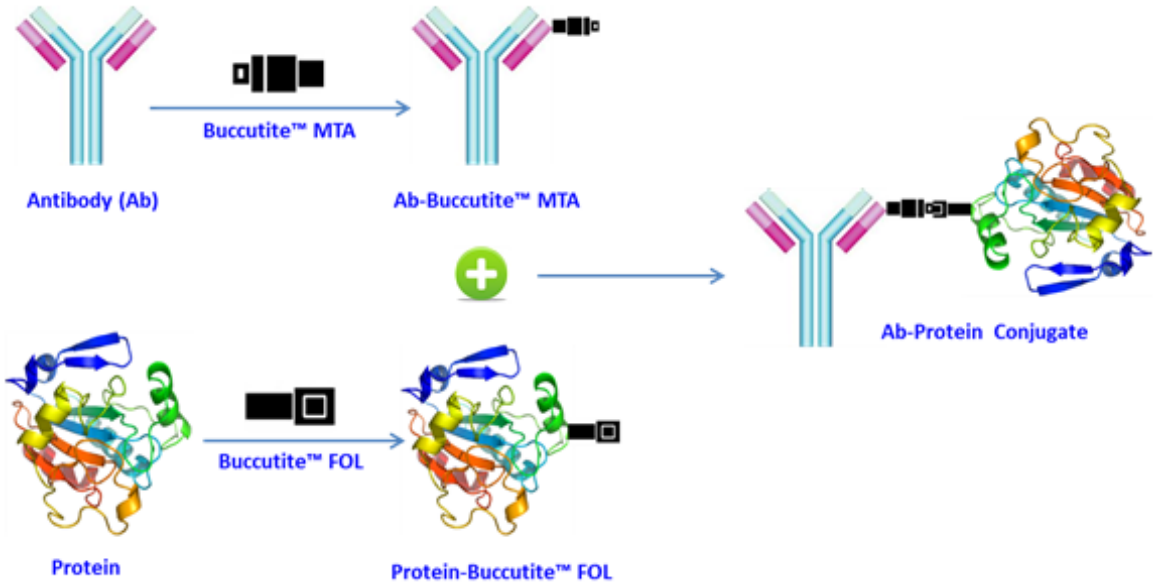

Figure 3.4. The work principle of Buccutite™ protein crosslinking technology.

Table 3.2 Buccutite™ Protein Crosslinking Technology

Cat #	Product Name	Size
5503	ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 µg Protein*	1 kit
5504	ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 kit
5507	ReadiLink™ Protein Crosslinking Kit *Optimized for 100 µg Protein*	1 kit
5508	ReadiLink™ Protein Crosslinking Kit *Optimized for 1 mg Protein*	1 kit

6-JOE SE

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

Compared to other fluorescein succinimidyl esters, 6-JOE SE generally gives much lower yield, and the resulted conjugates are also more difficult to be purified. Recently we have analyzed a number of commercial 6-JOE SE materials, and found that all the commercial 6-JOE SE materials contain a few impurities which often complicate the conjugations of 6-JOE SE with amino-modified oligonucleotides as shown in Figure 3.5. The impurities associated with the commercial 6-JOE SE materials include unreactive free 6-JOE acid, 3,6-JOE Bis SE and 3-JOE SE. In particular, the conjugate resulted from 3-JOE SE is difficult to be separated from the desired 6-JOE conjugate. 6-JOE SE offered by AAT Bioquest is essentially free of 3,6-JOE Bis SE and 3- JOE SE as shown in Figure 3.6.

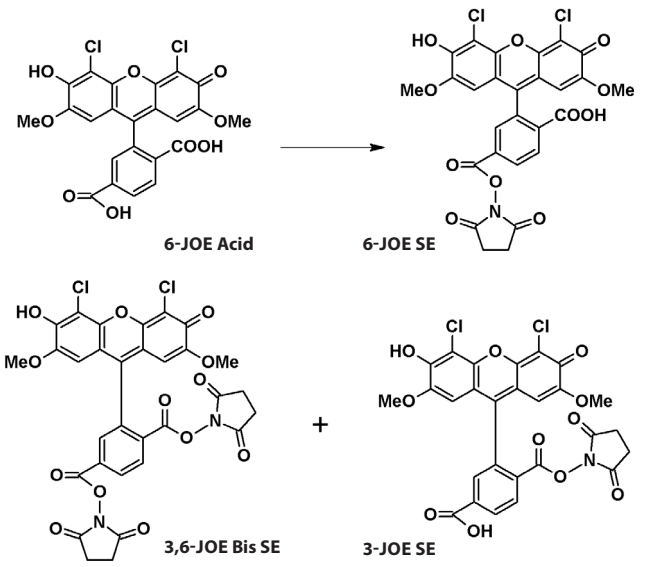

Figure 3.5. The reactions of 6-JOE, SE with amino-modified oligonucleotides are complicated by the impurities resulted from the manufacturing process of 6-JOE, SE.

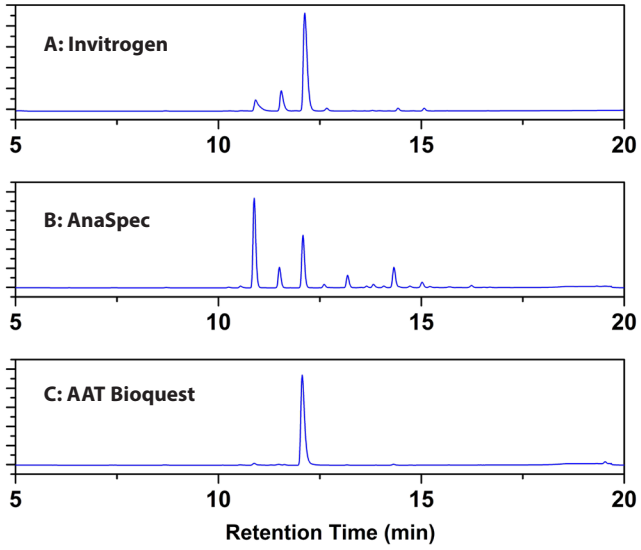

Figure 3.6. HPLC analysis of 6-JOE, SE materials from different commercial vendors. Vendor A: Invitrogen (Lot#: 1454485); Vendor B: AnaSpec (Lot#: 96700-1); Vendor C: AAT Bioquest (Lot#: 099133).

Table 3.3 6-JOE & Its Analogs for Labeling Oligonucleotides

Cat #	Product Name	Size	Ex (nm)	Em (nm)	EC ($cm^{-1} M^{-1}$)	CF @260 nm
241	6-HEX Alkyne	5 mg	533	550	74,000	0.300
240	6-HEX Azide	5 mg	533	550	74,000	0.300
6026	6-HEX Phosphoramidite [5'-Hexachlorofluorescein Phosphoramidite]	100 µmoles	533	550	74,000	0.300
202	6-HEX, SE [6-Carboxy-2',4,4',5',7,7'-hexachlorofluorescein, Succinimidyl Ester]	5 mg	533	550	74,000	0.300
249	6-JOE Alkyne	5 mg	520	548	73,000	0.326
248	6-JOE Azide	5 mg	520	548	73,000	0.326
203	6-JOE, SE [6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, Succinimidyl Ester]	5 mg	520	548	73,000	0.326
245	6-TET Alkyne	5 mg	521	536	76,000	0.191
244	6-TET Azide	5 mg	521	536	76,000	0.191
6027	6-TET Phosphoramidite [5'-Tetrachlorofluorescein Phosphoramidite]	100 µmoles	521	536	76,000	0.191
211	6-TET, SE [6-Carboxy-2',4,7',7-tetrachlorofluorescein, Succinimidyl Ester]	5 mg	521	536	78,000	0.191

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