Biomolecule Quantification Assays & Probes

BIOPOLYMERS · CELL METABOLITES · CELL SIGNALING MOLECULES

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Custom Products and Services

Our Technologies

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

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

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

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

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

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

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

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

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

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

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

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

Our Services

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

Custom Assay Design and Development

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

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

Custom Conjugation

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

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

Custom Screening

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

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

Custom Synthesis of Fluorophores and Luminophores

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

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Quantification of Functional Groups

2

2.1 Aldehyde Assays

Very reactive aldehydes, namely 4-hydroxyalkenals, were first shown to be formed in autoxidizing chemical systems. It was subsequently shown that 4-hydroxyalkenals, particularly 4-hydroxynonenal, were formed in substantial amounts under biological conditions, i.e. during the peroxidation of lipids of liver microsomes incubated in the NADPH-Fe system. Many other aldehydes, e.g., alkanals , alk-2-enals, and 4-hydroxyalkenals, were also identified in peroxidizing liver microsomes or hepatocytes.

The formation, reactivity and toxicity of aldehydes originating from the peroxidation of lipids of cellular membranes have received great attention in recent years. Rapid and accurate measurement of aldehydes is an important task for biological research, chemical research, food industry and environmental pollution surveillance. There are few reagents or assay kits available for quantifying the number of aldehydes. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS.

Both Amplite™ Colorimetric Aldehyde Quantitation Kits (Cat# 10051 & 10053) and Amplite™ Fluorimetric Aldehyde Quantitation Kit (Cat# 10052) have been developed for the rapid quantification of aldehydes. Amplite™ Colorimetric Aldehyde Quantitation Kits use a proprietary dye that generates a chromogenic product upon reacting with an aldehyde. Kit 10051 provides a sensitive, one-step colorimetric method to detect as little as 1 nanomole aldehyde in a 100 µL assay volume (10 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation without a separation step. Its signal can be easily read with an absorbance microplate reader at 405 nm or 550 nm. Kit 10051 has been used for monitoring activities of oxidases that convert an amino group to an aldehyde group.

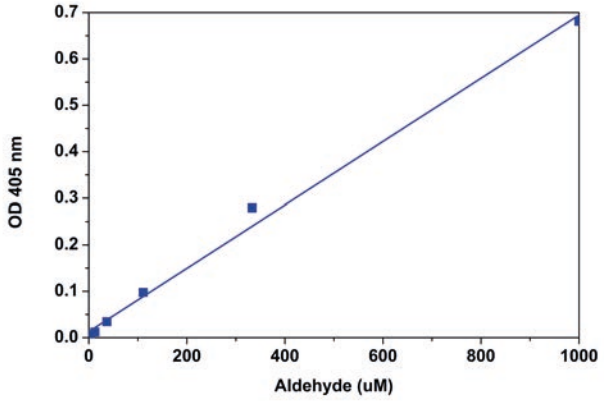

Figure 2.1. Aldehyde dose responses were measured with Amplite™ Colorimetric Aldehyde Quantitation Assay Kit (Cat# 10051) in a 96-well clear bottom plate. As low as 10 µM (1 nanomol/well) aldehyde was detected.

Amplite™ Fluorimetric Aldehyde Quantitation Kit (Cat# 10052) uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. Fluorimetric Kit 10052 is much more sensitive than colorimetric Kit 10051. The fluorimetric Kit 10052 provides a sensitive mix-and-read method to detect as little as 0.1 nanomole of aldehyde in a 100 µL assay volume (1 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 using a fluorescence microplate reader at Ex/Em = 365/435 nm.

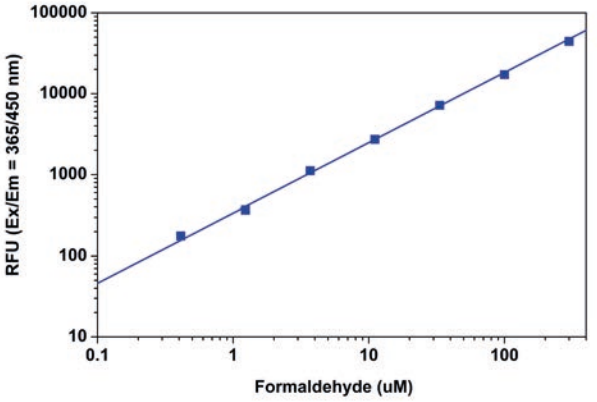

Figure 2.2. Formaldehyde dose responses were measured with Amplite™ Fluorimetric Aldehyde Quantitation Kit (Cat# 10052) in a 96-well black plate. As low as 1 µM (0.1 nanomol/well) formaldehyde was detected with 15 minutes incubation (n=3).

Table 2.1 Aldehyde Quantitation Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
10051	Amplite™ Colorimetric Aldehyde Quantitation Kit	200 tests	405 (550)	N/A
10053	Amplite™ Colorimetric Aldehyde Quantitation Kit *Blue Color*	200 tests	620	N/A
10052	Amplite™ Fluorimetric Aldehyde Quantitation Kit	200 tests	360	450

2.2 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 2.2 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.

Table 2.2 Selection Guide for Quantifying Maleimide Groups

Cat #	Detection Mode	Feature	Recommended Use
5523	Fluorescence	High sensitivity	Small molecules and soluble bioconjugates
5525	Absorption	Broad application	Nano particles and other materials
5526	Absorption	High accuracy	Proteins, oligos and nucleic acids

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 the maleimide-linked 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.

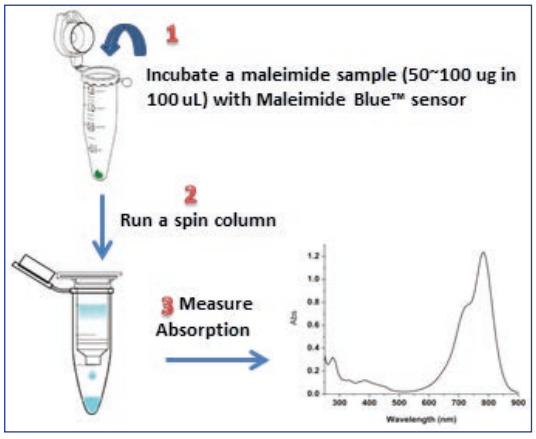

Figure 2.3. The overview of Amplite™ Rapid Colorimetric Maleimide Quantitation Kit (Cat# 5526).

Amplite™ Colorimetric Maleimide Quantitation Assay Kit (Cat# 5525) 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 $M^{-1}cm^{-1}$. 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.

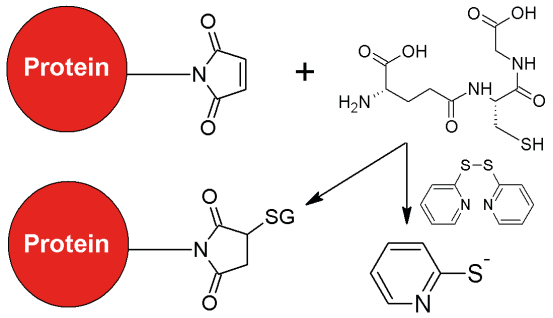

Figure 2.4. 4,4'-DTDP assay principle for quantifying maleimide.

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 and easily adapted to automation without a separation step. 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 suffers less interference from biological samples.

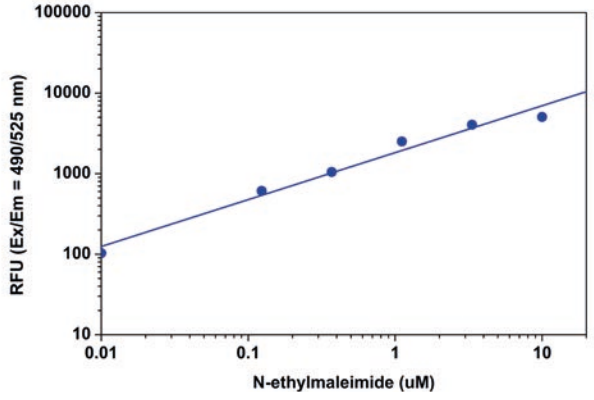

Figure 2.5. N-ethylmaleimide dose responses were measured with Amplite™ Fluorimetric Maleimide Quantitation Assay Kit (Cat# 5523) on a 96-well solid black plate. As low as 0.1 µM (10 pmol/well) N-ethylmaleimide was detected with 10 minutes incubation.

Table 2.3 Maleimide Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
5525	Amplite™ Colorimetric Maleimide Quantitation Kit	100 tests	324	N/A
5523	Amplite™ Fluorimetric Maleimide Quantitation Kit	200 tests	490	515
5526	Amplite™ Rapid Colorimetric Maleimide Quantitation Kit	2 tests	780	N/A

2.3 Thiol Detection Probes & Assays

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 quantifying thiol content in biological systems. All the commercial kits either lack sensitivity or have tedious protocols.

Thiolite™ Blue (Cat# 21507) is one of the most sensitive sensors for measuring thiol compounds. It gives a blue fluorescent adduct upon reacting with thiol compounds (such as GSH and cysteine). It can be used to quantifying the number of cysteines in a protein. We have used it to measure glutathione fluorimetrically. Thiolite™ Blue has >200-fold fluorescence enhancement upon reaction with thiol-containing compounds. Thiolite™ Blue is an excellent replacement for mBBr (monobromobimane) due to their similar spectral properties. Compared to mBBr, the thiol adduct of Thiolite™ Blue has much stronger fluorescence and absorption than those of mBBr, making it a much more sensitive thiol probe than bromobimanes. Thiolite™ Blue AM is optimized for intracellular thiol detection. It is non-fluorescent outside cells, eliminating the wash step and reducing assay background.

Besides Thiolite™ Blue, we also have developed Thiolite™ Green (Cat# 21508), another sensitive sensor for measuring thiol compounds. It gives a green fluorescent adduct upon reacting with thiol compounds (such as cysteine). The excitation and emission spectra of thiol-Thiolite™ Green adduct are similar to those of fluorescein, making Thiolite™ Green an excellent thiol detection probe that is compatible with almost all fluorescence instruments. The pH-independent excitation and emission wavelengths of Thiolite™ Green make Thiolite™ Green-based assays convenient and robust.

Amplite™ Fluorimetric Thiol Quantitation Assay Kit (Cat# 5524) provides an ultrasensitive fluorimetric assay to quantify thiol that exists either in a small molecule or in a protein. 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 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 signal can be easily read using a fluorescence microplate reader at Ex/Em = 490/520 nm. As little as 1 picomole cysteine or GSH in a 100 µL assay volume (10 nM) was detected using Amplite™ Fluorimetric Thiol Quantitation Assay Kit.

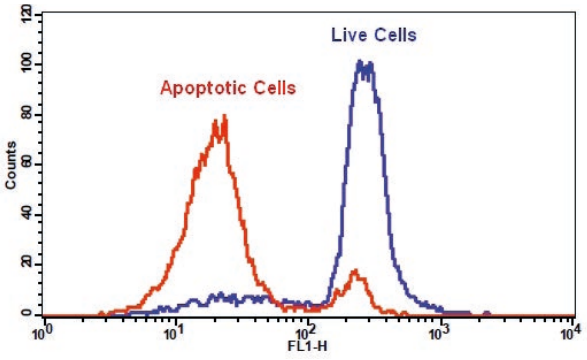

Figure 2.6. The detection of GSH in apoptotic cells with Cell Meter™ Intracellular GSH Assay Kit (Cat# 22810). Jurkat cells were treated overnight without (blue) or with 20 µM camptothecin (red) in a 37 °C, 5% CO_2 incubator, and loaded with Thiolite™ Green for 30 minutes. The fluorescence intensity of Thiolite™ Green was measured with a FACSCalibur™ flow cytometer using FL1 channel.

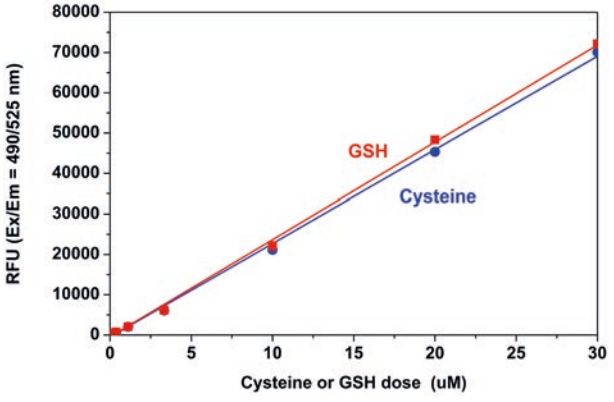

Figure 2.7. GSH and cysteine dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit (Cat# 5524) using a NOVOstar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) GSH or cysteine was detected with 10 minutes incubation (n=3).

Table 2.4 Thiol Detection Probes and Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
5524	Amplite™ Fluorimetric Thiol Quantitation Assay Kit *Green Fluorescence*	200 tests	510	524
22810	Cell Meter™ Intracellular GSH Assay Kit	100 tests	510	524
21507	Thiolite™ Blue	5 mg	335	460
21506	Thiolite™ Blue, AM	1 mg	335	460
21508	Thiolite™ Green	5 mg	510	524

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Quantification of Cell Metabolites & Cell Signaling Molecules

3

3.1 Acetylcholine (ACh) Assays

Acetylcholine (ACh) and its metabolites are involved in three main physiological purposes: structural integrity and signaling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and a major source for methyl groups via its metabolite. Acetylcholine is a neurotransmitter in both central and peripheral nervous systems. It is one of many neurotransmitters in the autonomic nervous system (ANS) and the only neurotransmitter used in the motor division of the somatic nervous system. It is involved in a number of biological events related to diabetic vasculopathy, hypertension, and Alzheimer's disease.

Amplite™ Fluorimetric Acetylcholine Assay Kit (Cat# 11403) provides one of the most sensitive methods for quantifying acetylcholine. The kit uses Amplite™ Red to quantify acetylcholine through the choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite™ Red is proportional to acetylcholine formation. The kit is an optimized "mix and read" assay. It provides an ultrasensitive one-step fluorimetric assay to detect as little as 0.01 nanomoles ACh in a 100 µL assay volume (0.1µM). Its signal can be easily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

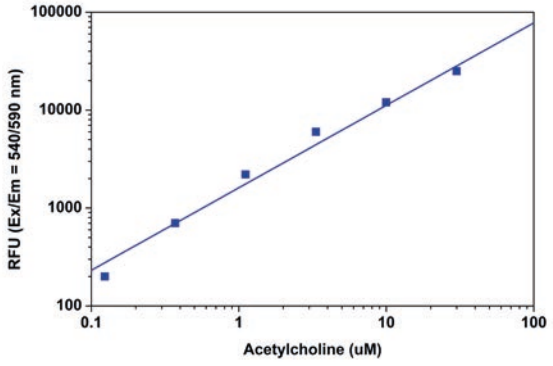

Figure 3.1. Acetylcholine dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Acetylcholine Assay Kit (Cat# 11403). As low as 0.01 nmoles/well (0.1µM) of acetylcholine was detected with 10 minutes incubation.

Table 3.1 Acetylcholine Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
11403	Amplite™ Fluorimetric Acetylcholine Assay Kit	200 tests	571	585

3.2 Adenosine Diphosphate (ADP) Assays

All signal transduction pathways are regulated on some level by phosphorylation, making phosphorylation relevant to most, if not all, areas of cell signaling and neuroscience research. Kinases are of interest to researchers involved in drug discovery due to their broad relevance to diseases. Most of the commercial protein kinase assay kits are either based on monitoring the phosphopeptide formation or ATP depletion. For the kinase assay kits based on the detection of phosphopeptides, one has to spend time and effort to identify an optimized peptide substrate. Detection of ADP formation is a very convenient method for monitoring kinase activities and phosphorylation process.

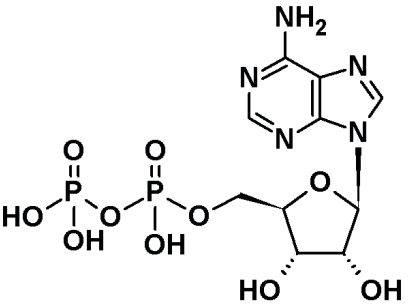

Figure 3.2. The chemical structure of adenosine diphosphate.

PhosphoWorks™ Fluorimetric ADP Assay Kit (Cat# 21655) is used for monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This kit provides a fast, simple, and homogeneous assay for the ADP measurement. It is a non-radioactive and no wash method to detect the amount of ADP produced as a result of enzyme activity. Its characteristics of high sensitivity (<0.3 µM ADP) and broad ATP tolerance (1-300 µM) make the kit ideal for determining kinase Michaelis-Menten kinetics as well as for screening and identifying kinase inhibitors.

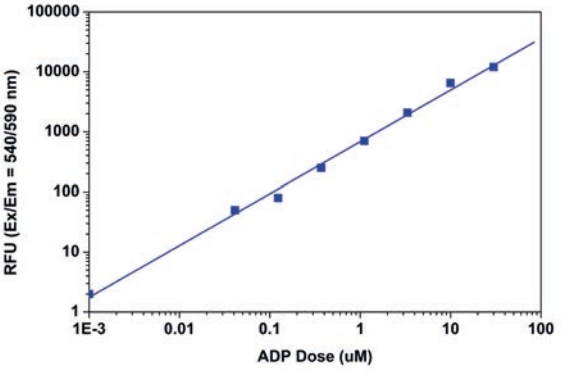

Figure 3.3. ADP dose responses were measured with PhosphoWorks™ Fluorimetric ADP Assay Kit (Cat# 21655) in a 384-well black solid plate. As low as 0.3 µM ADP was detected with 30 minutes incubation.

Table 3.2 Adenosine Diphosphate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
31001	Amplite™ Universal Fluorimetric Kinase Assay Kit *Red Fluorescence*	200 tests	571	585
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	100 tests	571	585

3.3 Adenosine Triphosphate (ATP) Assays

ATP plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The quantification of ATP can be used for a variety of biological applications. Because ATP is the energy source for almost all living organisms and rapidly degrades in the absence of viable organisms, its existence can be used to

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identify the presence of viable organisms. The measurement of ATP has been used for cell cytoxicity, detection of bacteria on surfaces, quantification of bacteria in water, somatic cells in culture and food quality.

Firefly luciferase is a monomeric 61 kD enzyme that catalyses a two-step oxidation of luciferin, which yields light at 560 nm. The first step involves the activation of the protein by ATP to produce a reactive mixed anhydride intermediate. In the second step, the active intermediate reacts with oxygen to create a transient dioxetane, which quickly breaks down to the oxidized product oxyluciferin and carbon dioxide along with a burst of light. When ATP is the limiting component, the intensity of light is proportional to the concentration of ATP. Thus the measurement of the light intensity can be used to quantify ATP with a luminometer.

PhosphoWorks™ Luminometric ATP Assay Kit 21610 comes with all the essential components and provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The assay is based on the detection of ATP using firefly luciferase to catalyze the release of light by ATP and luciferin. It can be performed in a convenient 96-well or 384-well microtiter-plate format and run with many luminescence instruments. The assay is extremely sensitive and can detect 10-100 cells/well. Its high sensitivity permits the detection of ATP in many biological systems, environmental samples and foods. Kit 21610 has higher sensitivity while Kit 21609 has more stable signal.

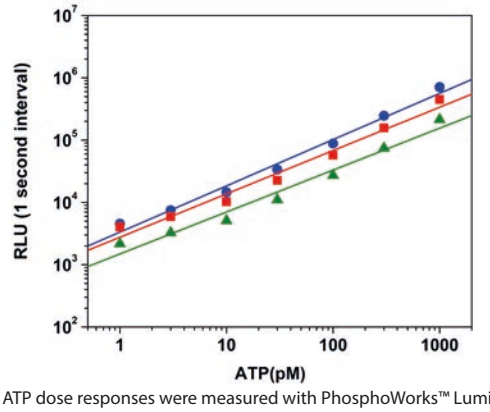

Figure 3.4. ATP dose responses were measured with PhosphoWorks™ Luminometric ATP Assay Kit (Cat# 21610) on a 96-well white plate. As low as 3 pM ATP was detected. The integration time was 1 second.

As Kit 21610, PhosphoWorks™ Luminometric ATP Assay Kit 21609 is also based on the detection of ATP using firefly luciferase to catalyze the release of light with ATP and luciferin. Complementary to Kit 21610, Kit 21609 provides a more stable luminescence signal that lasts for a few hours, making it convenient to be used with the luminometers that are not equipped with liquid handling capacity. Although Kit 21609 is less sensitive than Kit 21610, its more stable signal provides advantages for some particular applications, such as rapid diagnostic applications. Kit 21609 has been used successfully for rapid food safety inspection with a hand-held luminometer.

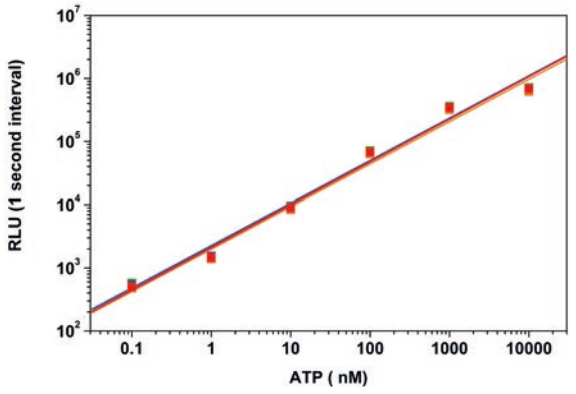

Figure 3.5. ATP dose responses were measured with PhosphoWorks™ Luminometric ATP Assay Kit (Cat# 21609). The linear luminescence signals for ATP concentrations from 100 µM to 0.1 nM were monitored for up to 5 hours (Z' factor = 0.7) without signal decay (The above figure shows 20 minutes, 1, 2, 3, 4, and 5 hours signal). The integrated time was 1 second.

PhosphoWorks™ Luminometric ATP Assay Kit 21612 is a DTT-free formulation and is extremely sensitive. 100 cells/well ATP was detected using Kit 21612. Its high sensitivity permits the detection of ATP in many biological systems, environmental samples and foods. It has stable luminescence signals with no mixing or separation required, and has been formulated to have minimal hands-on time.

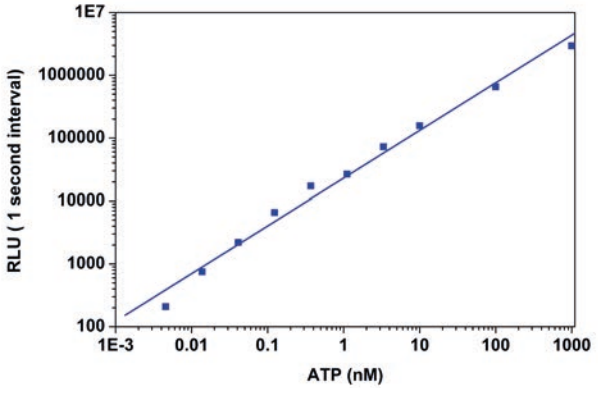

Figure 3.6. ATP dose responses were measured with PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free* (Cat# 21612) on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). 0.03 nM ATP was detected within 20 minutes. The integration time was 1 second. The half life was more than 2 hours.

Table 3.3 Adenosine Triphosphate Assay Kits

Cat #	Product Name	Size	Em (nm)
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	100 tests	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-free*	100 tests	560
21613	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-free*	1,000 tests	560
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	100 tests	560

3.4 L-Alanine Assays

L-alanine (L-Ala) plays a crucial role as a building block of important proteins. L-alanine is mostly synthesized by the muscle cells from lactic acid and absorbed into blood via liver. It is converted into pyruvate by glutamic-pyruvic transaminase to enter the metabolic mainstream. L-alanine is critical for the production of glucose and hence blood sugar management, and plays an important role in the immune system and the prevention of kidney stones. Insufficiency of L-alanine is usually a sign of poor nutrition, low protein diet, as well as stress.

Amplite™ Colorimetric L-Alanine Assay Kit (Cat# 13826) offers a sensitive colorimetric assay for quantifying L-alanine in biological samples. The kit utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by Quest Fluor™ L-Alanine Sensor at 575 nm. Amplite™ Fluorimetric L-Alanine Assay Kit (Cat# 13825) utilizes Quest Fluor™ L-Alanine Sensor as a fluorescent L-alanine indicator (Ex/Em = 540/590 nm).

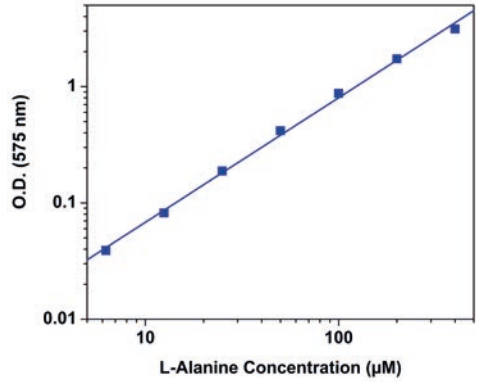

Figure 3.7. L-alanine dose responses were measured with Amplite™ Colorimetric L-Alanine Assay Kit (Cat# 13826) in a 96-well clear bottom plate. As low as 10 μM L-alanine was detected with 30 minutes incubation at 37 °C.

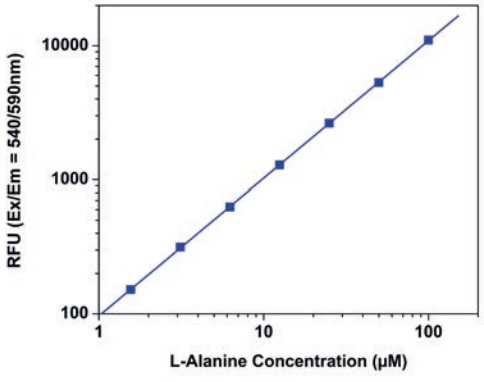

Figure 3.8. L-alanine dose responses were measured with Amplite™ Fluorimetric L-Alanine Assay Kit (Cat# 13825) in a 96-well black solid plate. As low as 1.5 μM L-alanine was detected with 30 minutes incubation at 37 °C.

3.5 Ammonia Assay

Ammonia is an important source of nitrogen for living systems. It is produced in liver and converted to urea through the urea cycle. Ammonia is synthesized through amino acid metabolism and is toxic when present at high concentrations. Elevated levels of ammonia in blood (hyperammonemia) have been found in liver dysfunction (cirrhosis), while hyperammonemia is associated with defects in the urea cycle enzymes (e.g. ornithine transcarbamylase). The determination of ammonia is very useful for monitoring health status.

Amplite™ Colorimetric Ammonia Quantitation Kit (Cat# 10059) provides a simple and sensitive colorimetric method for the quantification of ammonia in foods and biological samples, such as serum, plasma and urine, etc. The assay is based on an enzyme-coupled reaction of ammonia, which generates a blue colored product. The absorbance of the blue product is proportional to the concentration of ammonia, which can be measured colorimetrically at 660-670 nm. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

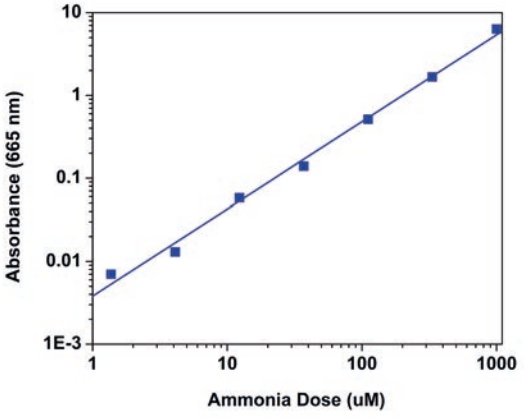

Figure 3.9. Ammonia dose responses were measured in a 96-well clear bottom plate with Amplite™ Colorimetric Ammonia Quantitation Kit (Cat# 10059). As low as 4 μM ammonia was detected (n=3) with 45 minutes incubation.

3.6 Ascorbic Acid Assay

Ascorbic acid (also called Vitamin C) is a critical metabolite for both plants and animals in cell division, growth and defense. Ascorbate is produced from glucose in the liver of most mammalian species. For humans, ascorbate has to be obtained from food to survive. A lack of sufficient vitamin C can result in scurvy, and may eventually lead to death. As an antioxidant ascorbate can reduce the risk of developing chronic diseases, such as cancer and cardiovascular disease. In food industry, ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives to prevent undesired color and taste.

Table 3.4 L-Alanine & Ammonia Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
10059	Amplite™ Colorimetric Ammonia Quantitation Kit *Blue Color*	200 tests	650	N/A
13826	Amplite™ Colorimetric L-Alanine Assay Kit	200 tests	575	N/A
13825	Amplite™ Fluorimetric L-Alanine Assay Kit	200 tests	571	585

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Amplite™ Fluorimetric Ascorbic Acid Assay Kit (Cat# 13835) offers a sensitive fluorescent assay for quantifying total ascorbic acid and the ratio of dehydroascorbate acid (DHA) to ascorbic acid in biological samples. The kit utilizes an enzyme reaction that oxidizes ascorbic acid to DHA, which can be detected by Ascorbrite™ Blue with a fluorescence microplate reader at Ex/Em = 340/430 nm. As low as 1 µM total ascorbic acid in a sample was detected.

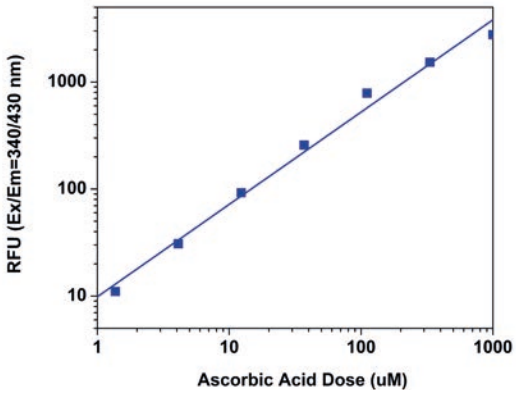

Figure 3.10. Ascorbic acid dose responses were measured with Amplite™ Fluorimetric Ascorbic Acid Assay Kit (Cat# 13835) on a 96-well black solid plate. As low as 1 µM ascorbic acid was detected with 30 minutes incubation.

3.7 Aspartate Assay

Aspartate (or aspartic acid) is a negatively charged, polar amino acid. Aspartate is involved in the control point of pyrimidine biosynthesis, in transamination reactions, in interconversions with asparagine, in the metabolic pathway leading to AMP, in the urea cycle, and is a precursor to homoserine, threonine, isoleucine, and methionine. It is also involved in the malate aspartate shuttle.

Amplite™ Aspartate Assay Kits offer a sensitive assay for quantifying aspartate in biological samples. In the assay, aspartate is converted to pyruvate and then utilizes an enzyme coupled reaction. The product hydrogen peroxide can be detected by Amplite™ Red sensor. The signal can be monitored using Amplite™ Colorimetric Aspartate Assay Kit (Cat# 13828) with an absorbance microplate reader at 575 nm. The signal can also be monitored using Amplite™ Fluorimetric Aspartate Assay Kit (Cat# 13827) with a fluorescence microplate reader at Ex/Em= 540/590 nm.

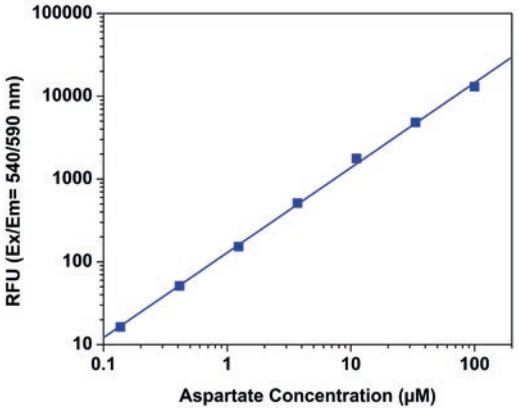

Figure 3.11. Aspartate dose responses were measured with Amplite™ Fluorimetric Aspartate Assay Kit (Cat#13827) on a 96-well black solid plate. As low as 0.4 µM aspartate was detected with 20-30 minutes incubation.

3.8 Biotin Assay

The avidin/streptavidin-biotin interaction is the strongest known non-covalent biological interaction ($K_d = 10^{-15}$ M^{-1}) between a protein and its ligand. One avidin binds four biotins. The bond formation between biotin and avidin/streptavidin is very rapid and, once formed, is unaffected by pH, organic solvents and other denaturing agents. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications.

Amplite™ Colorimetric Biotin Quantitation Kit (Cat# 5522) provides a convenient method for estimating the molar ratio of biotin to protein in biotin-protein conjugates or for quantifying biotin concentration in a solution. The assay uses HABA (4'-hydroxyazobenzene-2-carboxylic acid), a reagent that shows dramatic spectral changes when bound to avidin. Biotin easily displaces HABA from the HABA/avidin complex, resulting in a decrease of absorption at 500 nm. The kit provides an optimal ratio of avidin to HABA, and it is best used to determine biotin concentration in the range from 2 to 16 µM. The assay can be performed in a cuvette or microplate format.

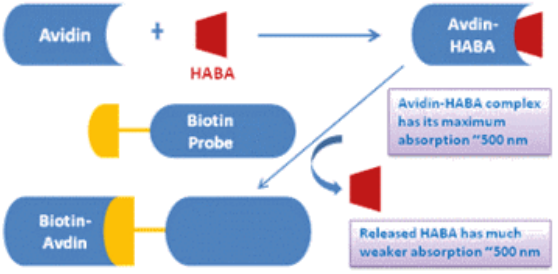

Figure 3.12. HABA assay principle for quantifying biotinylation degree.

Table 3.5 Ascorbic Acid, Aspartate & Biotin Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13828	Amplite™ Colorimetric Aspartate Assay Kit	200 tests	575	N/A
5522	Amplite™ Colorimetric Biotin Quantitation Kit	200 tests	500	N/A
13835	Amplite™ Fluorimetric Ascorbic Acid Assay Kit	200 tests	340	430
13827	Amplite™ Fluorimetric Aspartate Assay Kit	200 tests	571	585
5521	ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*	3 reactions	N/A	N/A

3.9 cAMP Assays

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 synthesized 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.

Screen Quest™ Colorimetric ELISA cAMP Assay kit (Cat# 36370) 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 adenylate cyclase activity in biochemical or cell-based assay system. Compared to other ELISA cAMP assay kits, Kit 36370 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.

Screen Quest™ FRET No Wash cAMP Assay Kit (Cat# 36379) provides another convenient assay method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. Compared to other commercial ELISA cAMP assay kits, this homogenous cAMP assay kit does not require a wash step or the acetylation step. The assay is based on the competition for a fixed number of anti-cAMP antibody binding sites between the fluorescent cAMP tracer and non-labeled free cAMP. Free cAMP displaces the fluorescent cAMP tracer from the HRP-cAMP/anti-cAMP antibody complex. The anti-cAMP antibody is labeled with our trFluor™ Tb while the cAMP tracer contains our bFluor™ 650. In the absence of cAMP, bFluor™ 650-cAMP conjugate is bound to trFluor™ Tb-labeled anti-cAMP antibody exclusively to have a strong FRET. However, the unlabeled free cAMP in the test sample competes for the trFluor™ Tb-labeled anti-cAMP antibody conjugate, therefore inhibits the binding of bFluor™ 650-cAMP to anti-cAMP antibody. The bFluor™ 650 labeled cAMP tracer only has fluorescence lifetime of nanosecond while trFluor™ Tb-labeled anti-cAMP antibody-bound fluorescent cAMP tracer has much longer fluorescence lifetime value due to the TR-FRET. The magnitude of FRET is proportional to the concentration of cAMP in a sample.

Screen Quest™ Fluorimetric ELISA cAMP Assay Kit (Cat# 36373) provides a sensitive method for detecting adenylate 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.

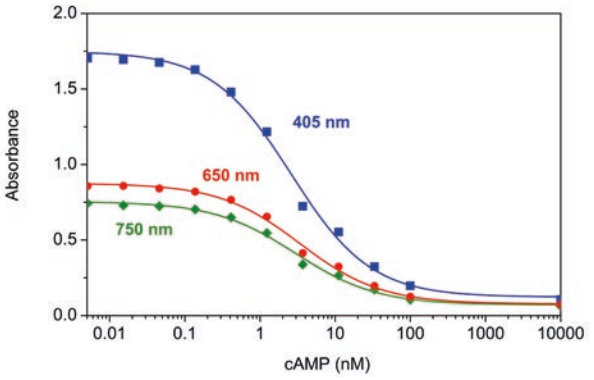

Figure 3.13. cAMP dose responses were measured with Screen Quest™ Fluorimetric ELISA cAMP Assay Kit (Cat# 36373) in a 96-well black solid plate. As low as 0.1 nM of cAMP was detected in a 100 µL reaction volume.

Screen Quest™ Live Cell cAMP Assay Service Pack (Cat# 36382) 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, $G_{\alpha 16}$. 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 $G_{\alpha 16}$ with specific non-G_q-coupled receptors results 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.

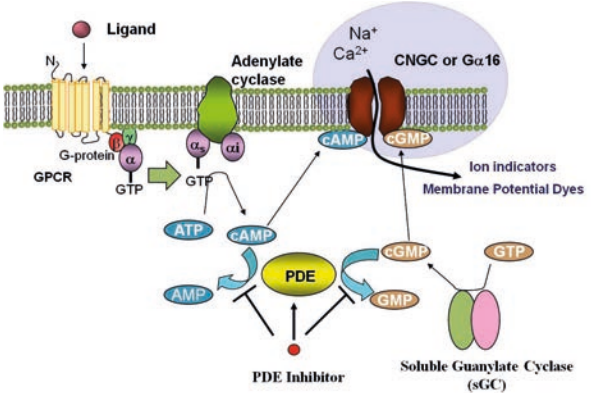

Figure 3.14. Screen Quest™ Live Cell cAMP Assay Principle (Cat# 36382).

Table 3.6 cAMP Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
36370	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	100 tests	650	N/A
36373	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	100 tests	571	585
36379	Screen Quest™ FRET No Wash cAMP Assay Kit	100 tests	390	650
36380	Screen Quest™ FRET No Wash cAMP Assay Kit	1,000 tests	390	650
36382	Screen Quest™ Live Cell cAMP Assay Service Pack	each	490	520

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3.10 Cholesterol Assay

Cholesterol is required to build and maintain cell membranes. It modulates membrane fluidity over the range of physiological temperatures. Within cells, cholesterol is the precursor molecule in several biochemical pathways. Cholesterol is also an important precursor molecule for the synthesis of vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone as well as the sex hormones progesterone, estrogens, together with testosterone and their derivatives.

Amplite™ Cholesterol Quantitation Assay Kit (Cat# 40006) provides one of the most sensitive methods for quantifying cholesterol. The kit uses Amplite™ Red to quantify the concentration of cholesterol, which is related to the production of hydrogen peroxide in the cholesterol oxidase-mediated enzyme coupling reactions. In the presence of peroxidase, the fluorescence intensity of Amplite™ Red is proportional to the concentration of hydrogen peroxide converted to the concentration of cholesterol. The assay can be readily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm.

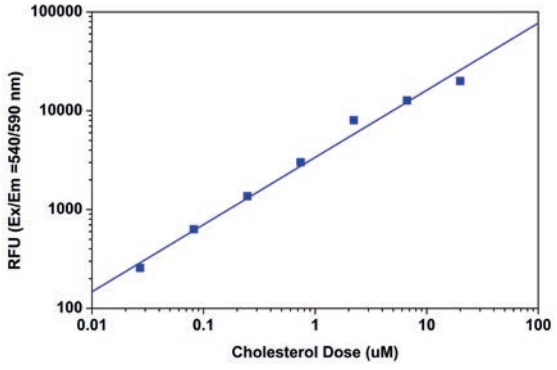

Figure 3.15. Cholesterol dose responses measured with Amplite™ Cholesterol Quantitation Assay Kit (Cat# 40006) on a 96-well black solid plate. As low as 0.03 µM cholesterol was detected with 30 minutes incubation (n=3).

3.11 Choline Assay

Choline and its metabolites play an important role in the structural integrity and signaling of cell membranes and cholinergic neurotransmission (acetylcholine synthesis). Choline is a major source for methyl groups via its metabolite, trimethylglycine that participates in the S-adenosylmethionine synthesis pathways. Choline deficiency may cause liver disease, atherosclerosis and possibly neurological disorders. Despite its importance in the central nervous system as a precursor for acetylcholine and membrane phosphatidylcholine, the role of choline in mental illness has been little studied.

Amplite™ Choline Quantitation Kit (Cat# 40007) provides one of the most sensitive methods for quantifying choline. The kit uses Amplite™ Red to quantify the concentration of choline, which is related to the production of hydrogen peroxide in the choline oxidase-mediated enzyme coupling reactions. The amount of choline is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. In the presence of peroxidase, the fluorescence intensity of Amplite™ Red is proportional to the formation of hydrogen peroxide converted to the concentration of choline. The assay can be readily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm. Alternatively the assay can also be read at ~570 nm with an absorbance microplate reader.

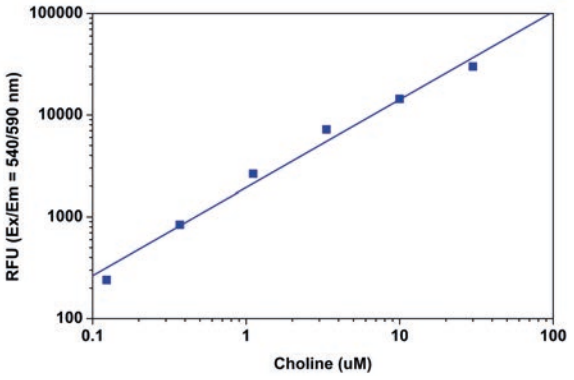

Figure 3.16. Choline dose responses were measured with Amplite™ Choline Quantitation Kit (Cat# 40007) in a 96-well solid black plate. As low as 0.1 µM (10 picomoles/well) choline was detected with 10 minutes incubation (n=3).

3.12 Coenzyme A (CoA) Assay

Coenzyme A (CoA) is a universal and essential cofactor in all forms of cellular life acting as a principal acyl carrier in numerous biosynthetic, energy-yielding, and degradative pathways. It plays important roles in the synthesis and oxidation of fatty acids, pyruvate oxidation and the citric acid cycle. Measurement of CoA 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 quantifying CoA content in biological systems. The existing commercial kits either lack sensitivity or have tedious procedures. Amplite™ Fluorimetric CoA Quantitation Assay Kit (Cat# 15270) provides an ultrasensitive fluorimetric assay to quantify CoA content by the detection of –SH group in CoA. Our proprietary fluorogenic CoA Green™ dye used in the kit becomes strongly fluorescent upon reacting with –SH. As little as 4 picomole of CoA in a 100 µL assay volume (40 nM) was detected. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format at Ex/Em = 490/520 nm.

Table 3.7 Cholesterol, Choline and Coenzyme A Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
40006	Amplite™ Cholesterol Quantitation Assay Kit	200 tests	571	585
40007	Amplite™ Choline Quantitation Kit	200 tests	571	585
15270	Amplite™ Fluorimetric Coenzyme A Quantitation Kit *Green Fluorescence*	200 tests	510	524

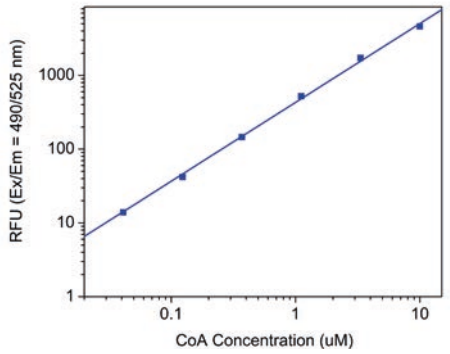

Figure 3.17. CoA dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Coenzyme A Quantitation Assay Kit (Cat# 15270). As low as 40 nM (4 pmol/well) CoA was detected with 30 minutes incubation (n=3).

3.13 Ethanol Assay

Ethanol is a powerful psychoactive drug and one of the oldest recreational drugs. It is best known as the type of alcohol found in alcoholic beverages and thermometers. In common usage, it is often simply referred to as alcohol or spirits. Ethanol is a central nervous system depressant and has significant psychoactive effects in sublethal doses. A blood ethanol level of 0.5% or more is commonly fatal. Ethanol levels of even less than 0.1% can cause intoxication with unconsciousness often occurring at 0.3–0.4%. The amount of ethanol in the body is typically quantified by blood alcohol content.

The ability to rapidly perform quantitative measurements of ethanol is highly desirable in life science research, clinical evaluations, food, and pharmaceutical industries. Our non-radioactive ethanol assay is based on the oxidation of ethanol by alcohol oxidase. Amplite™ Fluorimetric Ethanol Quantitation Kit (Cat# 40001) uses our Amplite™ Red reagent that makes the kit recordable in a dual mode, the fluorescent signal can be easily read by a fluorescence microplate reader at Ex/Em = ~540/590 nm, or its absorption can be readily read by an absorbance microplate reader at ~570 nm. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format. The assay can be completed within 30 minutes and as little as 0.0003% ethanol can be detected.

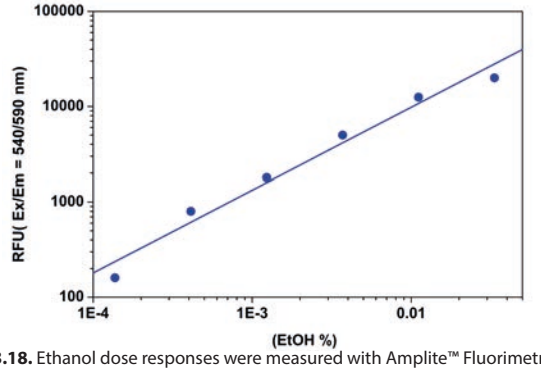

Figure 3.18. Ethanol dose responses were measured with Amplite™ Fluorimetric Ethanol Quantitation Kit (Cat# 40001) on a 96-well black solid plate. As low as 0.0003% ethanol was detected with 15 minutes incubation (n=3).

3.14 Fatty Acid Uptake Assay

Fatty acid uptake is an important therapeutic target for the treatment of many human diseases, such as obesity, type 2 diabetes and hepatic steatosis. Screen Quest™ Fluorimetric Fatty Acid Uptake Assay Kit (Cat# 36385) provides a simple and sensitive method for the measurement of fatty acid uptake in cells containing fatty acid transporters.

Screen Quest™ Fluorimetric Fatty Acid Uptake Assay Kit (Cat# 36385) uses a proprietary dodecanoic acid fluorescent fatty acid substrate. The assay can be performed in a convenient 96-well or 384-well microtiter plates and readily adapted to high throughput screening applications without a separation step. Its signal can be easily read using any fluorescence microplate reader with the bottom-read mode at Ex/Em = 485/515 nm or in FITC channel.

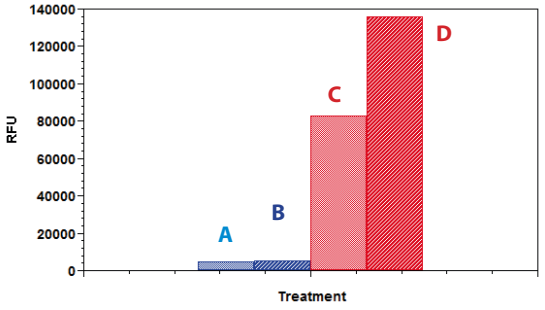

Figure 3.19. Comparison between fatty acid uptake in 3T3-L1 adipocytes and fatty acid uptake in 3T3-L1 fibroblasts. Cells were plated at 50,000 cells/100 µL/well in a 96 well black wall/clear bottom poly-D lysine plate for 5 hours, and then serum was deprived for 1 hour. Cells were treated without (control) or with insulin (150 nM), and incubated in a 5% CO_2 incubator at 37 °C for 30 minutes. At the end of the incubation, 100 µL of fatty acid mixture was added into the well, and incubated for another 60 minutes. The fluorescence signals were measured with a FlexStation® plate reader using bottom read mode. A: fibroblasts (Control); B: fibroblasts (Insulin); C: adipocytes (Control); D: adipocytes (Insulin).

3.15 Formaldehyde Assay

Formaldehyde is a naturally occurring substance. Natural processes in the upper atmosphere may contribute up to 90 percent of the total formaldehyde in the environment. Formaldehyde, as well as its oligomers and hydrates are rarely encountered in living organisms. Methanogenesis proceeds via the equivalent of formaldehyde, but this one-carbon species is masked as a methylene group in methanopterin. Formaldehyde is the primary cause of methanol's toxicity, since methanol is metabolized into toxic formaldehyde by alcohol dehydrogenase.

Amplite™ Fluorimetric Formaldehyde Quantitation Kit (Cat# 10057) provides a sensitive mix-and-read method to detect formaldehyde. The kit uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with formaldehyde. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without

Table 3.8 Ethanol and Fatty Acid Uptake Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
40001	Amplite™ Fluorimetric Ethanol Quantitation Kit	200 tests	571	585
36385	Screen Quest™ Fluorimetric Fatty Acid Uptake Assay Kit	200 tests	571	585

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a separation step. Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 400/510 nm.

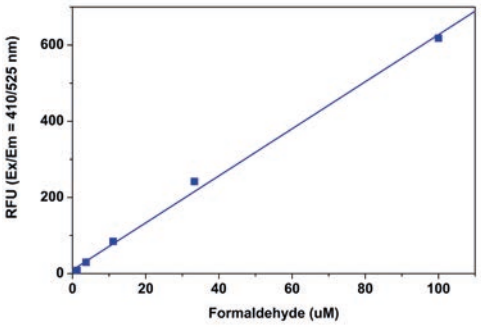

Figure 3.20. Formaldehyde dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Formaldehyde Quantitation Kit (Cat# 10057). As low as 1 µM formaldehyde was detected with 30 minute incubation.

Table 3.9 Formaldehyde Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
10057	Amplite™ Fluorimetric Formaldehyde Quantitation Kit	200 tests	400	510

3.16 Glucose Uptake Assays

Glucose transport systems are responsible for transporting glucose across cell membranes. Measuring the uptake of 2-deoxyglucose (2-DG), a glucose analog, is widely accepted as a reliable method to estimate the amount of glucose uptake and to investigate the regulation of glucose metabolism and insulin resistance. The 2-DG uptake is commonly determined using the non-metabolized 2-DG labeled with tritium or C^{14}. However, the radiolabeled probe is costly and requires a tedious special handling procedure.

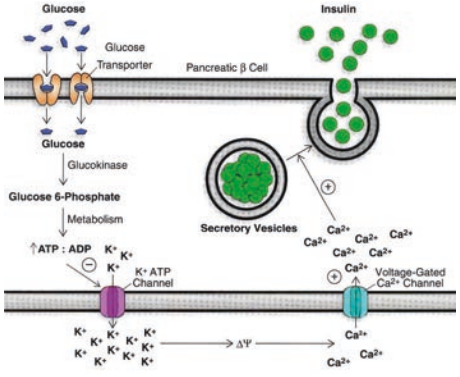

Figure 3.21. Glucose transportation into cells via glucose transporters, Glu 1, Glu 2, Glu 3 and Glu 4.

Screen Quest™ Colorimetric Glucose Uptake Assay Kit (Cat# 36503) provides a sensitive and non-radioactive glucose uptake assay. In this assay, 2-DG is taken up by glucose transporters, and metabolized to 2-DG-6-phosphate (2-DG6P). The non-metabolizable 2-DG6P accumulates in cells and is proportional to glucose uptake. The accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a chromogenic NADPH sensor. The signal can be read using an absorbance microplate reader by reading the OD ratio of 570 nm to 610 nm. Based on the same principle, Screen Quest™ Fluorimetric Glucose Uptake Assay Kit (Cat# 36500) provides an even more sensitive glucose uptake assay. The accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a fluorogenic NADPH sensor. The signal can be read using a fluorescence microplate reader at Ex/Em = 540 nm/590 nm.

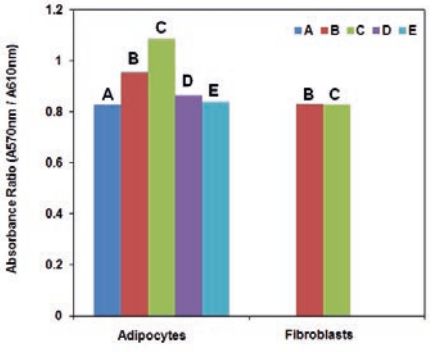

Figure 3.22. Measurements of 2-DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Colorimetric Glucose Uptake Assay Kit (Cat# 36503) in a 96-well black wall/clear bottom cell culture Poly-D-Lysine plate. (A: Negative Control, no insulin and no 2-DG treatment. B: 2-DG uptake in the absence of insulin. C: 2-DG uptake in the presence of 1µM insulin. D: 2-DG uptake in the presence of 1µM insulin and 200 µM phloretin. E: 2-DG uptake in the presence of 1µM insulin and 5 mM D-glucose.)

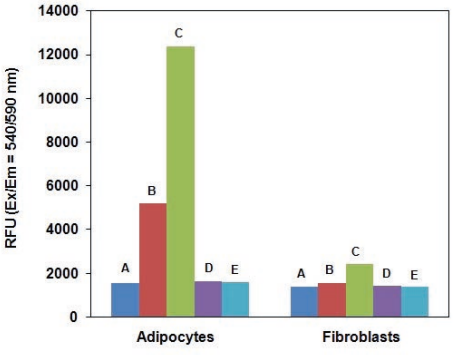

Figure 3.23. Measurements of 2-DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Fluorimetric Glucose Uptake Assay Kit (Cat# 36500) in a 96-well black wall/clear bottom cell culture Poly-D-Lysine plate. A: Negative Control, no insulin and no 2-DG treatment; B: 2-DG uptake in the absence of insulin; C: 2-DG uptake in the presence of 1 µM insulin; D: 2-DG uptake in the presence of 1 µM insulin and 200 µM phloretin; E: 2-DG uptake in the presence of 1 µM insulin and 5 mM D-glucose.

Table 3.10 Glucose Uptake Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
36503	Screen Quest™ Colorimetric Glucose Uptake Assay Kit	100 tests	575	N/A
36504	Screen Quest™ Colorimetric Glucose Uptake Assay Kit	500 tests	575	N/A
36500	Screen Quest™ Fluorimetric Glucose Uptake Assay Kit	100 tests	571	585
36501	Screen Quest™ Fluorimetric Glucose Uptake Assay Kit	500 tests	571	585

3.17 Glucose Assays

Glucose, a monosaccharide, is the most important carbohydrate in biology. It is a source of energy and metabolic intermediate for cell growth. As one of the main products of photosynthesis, glucose starts cellular respiration in both prokaryotes and eukaryotes. Glucose level is a key diagnostic parameter for many metabolic disorders, e.g., diabetes.

Amplite™ Glucose Quantitation Kits (Cat# 40004 and 40005) provide a quick and sensitive method for the measurement of glucose. They use glucose oxidase-based enzyme coupled reactions to detect glucose through the production of hydrogen peroxide, which is monitored by our Amplite™ Red peroxidase substrate. Amplite™ Red peroxidase substrate can be recorded in a dual mode, the fluorescence signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm, and its absorption can be read by an absorbance microplate reader at ~570 nm. The assay is robust, and can be readily adapted for a wide variety of applications that require the measurement of glucose. The assay runs in the red visible range that significantly reduces the interference from biological samples. It has demonstrated high sensitivity and low interference with excitation at 570 nm and emission at 590 nm. With Amplite™ Fluorimetric Glucose Quantitation Kit (Cat# 40005), as little as 0.1 µM glucose was detected. With Amplite™ Colorimetric Glucose Quantitation Kit (Cat# 40004), as little as 3 µM glucose was detected.

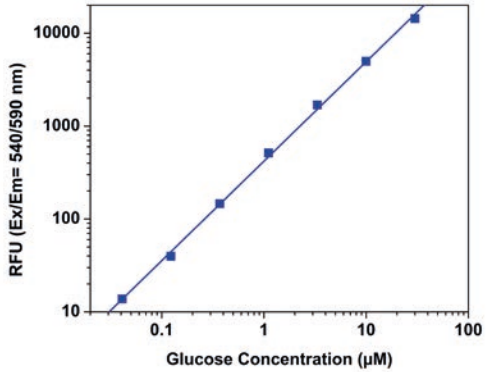

Figure 3.24. Glucose dose responses were measured with Amplite™ Fluorimetric Glucose Quantitation Kit (Cat# 40005) on a 96-well black solid plate. As low as 0.1 µM glucose was detected with 30 minutes incubation.

3.18 Glucose -6-Phosphate (G6P) Assays

Glucose-6-phosphate (G6P) is a key intermediate for glucose to transport into cells. G6P may also be converted to glycogen or starch mainly stored in liver and muscle cells. G6P is utilized by glucose-6-phosphate dehydrogenase (G6PD) to generate the reducing equivalents in the form of NADPH. This is particularly important in red blood cells where G6PD deficiency leads to hemolytic anemia.

Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit (Cat# 13804) provides a simple, sensitive and rapid fluorescence-based method for detecting G6P in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the coupled enzyme assay, the G6P concentration is proportionally related to NADPH that is specifically monitored by a fluorogenic NADPH sensor. The fluorescence signal can be read using a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (Ex/Em = 540 nm/590 nm is recommended). With Amplite™ Fluorimetric G6P Assay Kit, as low as 0.3 µM G6P was detected.

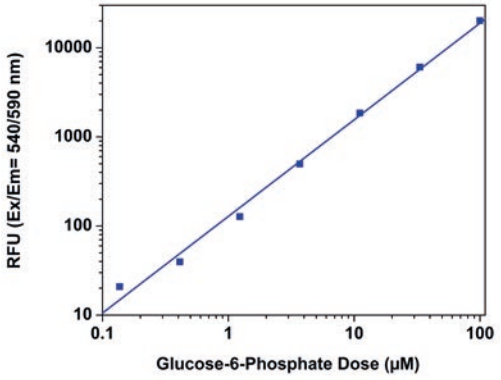

Figure 3.25. G6P dose responses were measured with Amplite™ Fluorimetric G6P Assay Kit (Cat# 13804) in a 96-well black plate. As low as 0.3 µM G6P was detected with 1 hour incubation.

Amplite™ Colorimetric Glucose-6-Phosphate Assay Kit (Cat# 13805) provides a simple, sensitive and rapid absorbance-based method for detecting G6P in biological samples. In the coupled enzyme assay, the G6P concentration is proportionally related to NADPH that is specifically monitored by a chromogenic NADPH sensor. The absorption signal can be read by an absorbance microplate reader at ~575 nm. With Amplite™ Colorimetric G6P Assay Kit, as little as 1 µM G6P was detected.

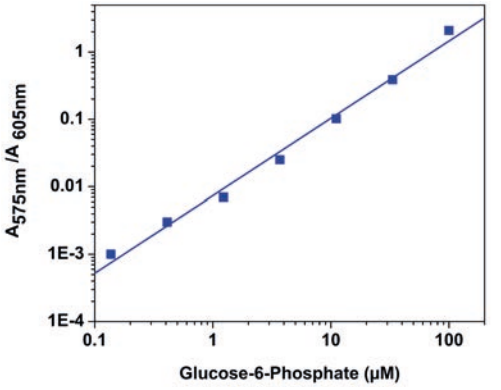

Figure 3.26. G6P dose responses were measured with Amplite™ Colorimetric G6P Assay Kit (Cat# 13805) on a 96-well white clear bottom plate. As low as 1 µM G6P was detected with 1 hour incubation.

Table 3.11 Glucose and Glucose -6-Phosphate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13805	Amplite™ Colorimetric Glucose-6-Phosphate Assay Kit	200 tests	575	N/A
40004	Amplite™ Colorimetric Glucose Quantitation Kit	500 tests	575	N/A
13804	Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit	200 tests	571	585
40005	Amplite™ Fluorimetric Glucose Quantitation Kit	500 tests	571	585

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3.19 Glutamic Acid Assay

Glutamic acid is one of the 20 proteinogenic amino acids. The carboxylate anions and salts of glutamic acid are known as glutamates. Glutamate is an important neurotransmitter which plays a key role in long-term potentiation and is important for learning and memory. Glutamic acid is the precursor of GABA but has somewhat the opposite function; it might play a role in the normal function of the heart and the prostate. As one of the few nutrients that cross the blood-brain barrier, glutamic acid is used in the treatment of diseases such as depression, ADD and ADHD, fatigue, alcoholism, epilepsy, muscular dystrophy, mental retardation, and schizophrenia.

Amplite™ Fluorimetric Glutamic Acid Assay Kit (Cat# 10054) provides a quick and sensitive method for the measurement of glutamic acid in various biological samples. In the assay, the coupled enzyme system catalyzes the reaction between L-glutamic acid and NADP to produce NADPH, which is specifically recognized by NADPH sensor and recycled back to NADP. A red fluorescent product is produced during the reaction. The signal can be read using either a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576 ± 5 nm. With Amplite™ Fluorimetric Glutamic Acid Kit, as little as 1 µM glutamic acid was detected. The assay is robust, and can be readily adapted for a wide variety of applications that require the measurement of glutamic acid.

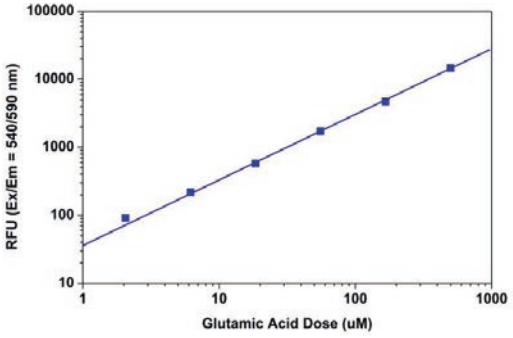

Figure 3.27. Glutamic acid dose responses were measured with Amplite™ Fluorimetric Glutamic Acid Assay Kit (Cat# 10054) in a 96-well black solid plate. As low as 1 µM glutamic acid was detected with 1 hour incubation.

3.20 Glutathione (GSH) Assay

Glutathione (GSH) is a tripeptide that contains L-cysteine, L-glutamic acid, and glycine. It is the smallest intracellular protein thiol molecule in cells, which regulates cell activity and prevents damages caused by reactive oxygen species such as free radicals and peroxides. The monitoring of reduced and oxidized GSH in biological samples is necessary for evaluating the redox and detoxification status of the cells and tissues against oxidative and free radicals mediated cell injury. The detection and measurement of glutathione 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 quantifying glutathione content in biological systems, but all the commercial kits either lack sensitivity or have tedious protocols.

Amplite™ Fluorimetric Glutathione Assay Kit (Cat# 10055) provides an ultrasensitive fluorimetric assay to quantify GSH in a sample. The proprietary non-fluorescent glutathione sensor used in the kit becomes strongly green fluorescent upon reacting with a GSH compound, which has the spectral properties almost identical to those of fluorescein and can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. As little as 1 picomole of GSH in a 100 µL assay volume (10 nM) was detected. In addition, both absorption and emission spectra of the glutathione adduct are pH-independent, making this assay kit highly robust.

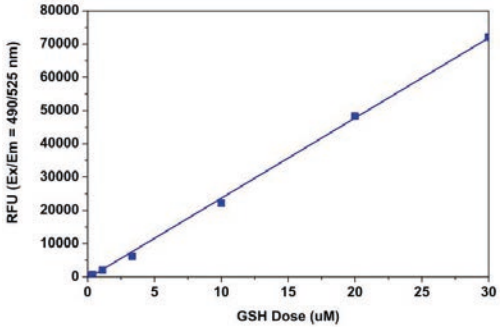

Figure 3.28. GSH dose responses were measured with Amplite™ Fluorimetric Glutathione Assay Kit (Cat# 10055) on a 96-well black solid plate. As low as 10 nM (1 pmol/well) GSH was detected with 10 minutes incubation.

3.21 Glutathione GSH/GSSG Ratio Assay

Glutathione exists in reduced (GSH) and oxidized (GSSG) states. Reduced glutathione (GSH) is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water. In the GPx catalyzed reaction, the formation of a disulfide bond between two GSH molecules generates oxidized glutathione (GSSG). The enzyme glutathione reductase (GR) recycles GSSG to GSH with the simultaneous oxidation of β-nicotinamide adenine dinucleotide phosphate (β-NADPH2). In healthy cells, more than 90% of the total glutathione pool is in the reduced form (GSH). When cells are exposed to increased levels of oxidative stress, GSSG accumulates and the ratio of GSSG to GSH increases. An increased ratio of GSSG-to-GSH is an indication of oxidative stress.

The monitoring of reduced and oxidized GSH in biological samples is essential for evaluating the redox and detoxification status of the cells and tissues against oxidative and free radicals mediated cell injury. There are quite a few reagents and assay kits available for quantifying thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols. Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat# 10056)

Table 3.12 Glutamic Acid, Glutathione & GSH/GSSG Ratio Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
10054	Amplite™ Fluorimetric Glutamic Acid Assay Kit *Red Fluorescence*	200 tests	571	585
10055	Amplite™ Fluorimetric Glutathione Assay Kit *Green Fluorescence*	200 tests	510	524
10056	Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit *Green Fluorescence*	200 tests	510	524

provides an ultrasensitive assay to quantify GSH in a sample. The kit uses a proprietary non-fluorescent dye that becomes strongly fluorescent upon reacting with GSH. With a one-step fluorimetric method, as little as 1 picomole of GSH or GSSG in a 100 µL assay volume was detected.

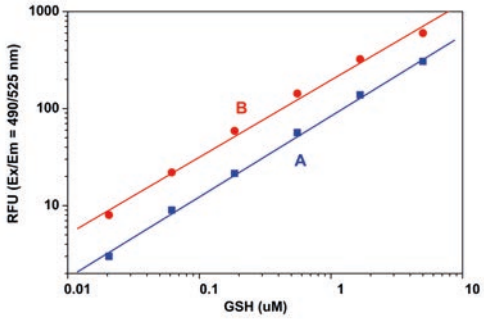

Figure 3.29. GSH and Total GSH (GSH+GSSG) dose responses were measured with Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat# 10056). Blue line (A): in the presence of GSH only; Red line (B), in the presence of 1:1 GSH/GSSG.

3.22 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.

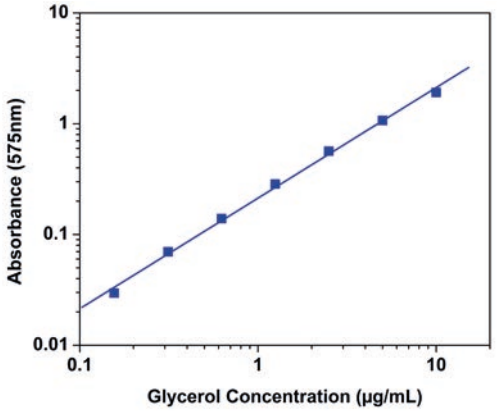

Figure 3.30. Glycerol dose responses were measured with Amplite Colorimetric Glycerol Assay Kit (Cat# 13832) on a 96-well black wall/clear bottom plate. As low as 0.15 µg/mL (~1.6 µM) glycerol was detected with 30 minutes incubation.

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.

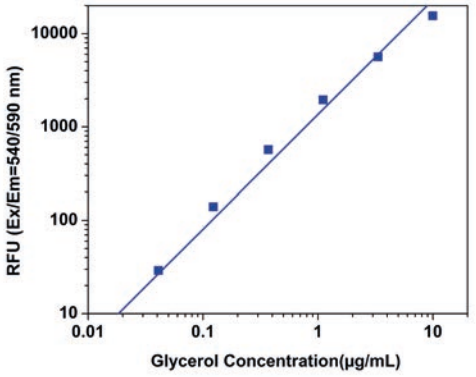

Figure 3.31. Glycerol dose responses were measured with Amplite™ Fluorimetric Glycerol Assay Kit (Cat# 13833) on a 96-well black solid plate. As low as 0.015 µg/mL (~0.16 µM) glycerol was detected with 20 minutes incubation.

Table 3.13 Glycerol Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13832	Amplite™ Colorimetric Glycerol Assay Kit	200 tests	575	N/A
13833	Amplite™ Fluorimetric Glycerol Assay Kit	200 tests	571	585

3.23 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 & 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.

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

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

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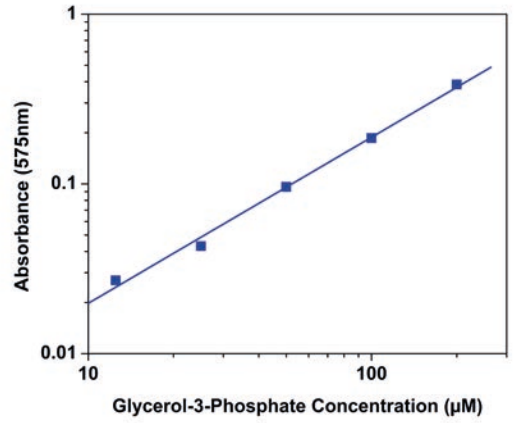

Figure 3.32. Glycerol 3-phosphate dose responses were obtained with Amplite™ Colorimetric Glycerol 3-Phosphate Assay Kit (Cat#13838) on a 96-well clear bottom plate. As low as 12.5 μM glycerol 3-phosphate was detected with 30 minutes incubation.

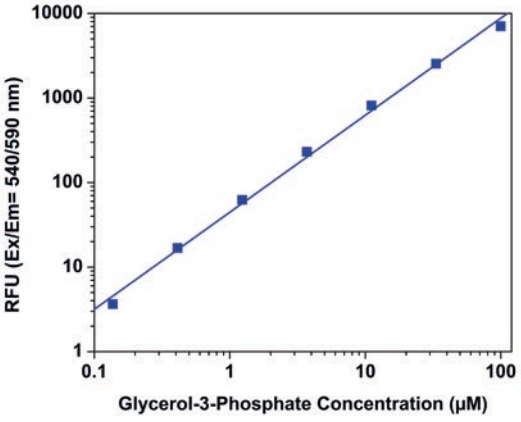

Figure 3.33. Glycerol 3-phosphate dose responses were obtained with Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat#13837) on a 96-well black solid plate. As low as 0.41 μM glycerol 3-phosphate was detected.

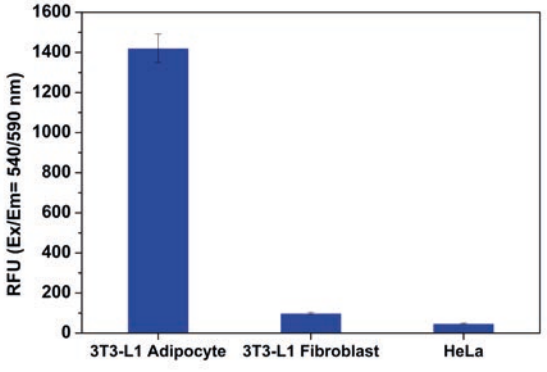

Figure 3.34. Measurements of glycerol 3-phosphate in 3T3-L1 adipocyte, 3T3-L1 fibroblast and HeLa cell lysates using Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat#13837) . Cells (1×10^5) were lysed using ReadiUse™ Mammalian Cell Lysis Buffer (Cat# 20012), and then 50 μL of cell lysate was used as glycerol 3-phosphate containing test samples. Assays were performed following the kit protocol.

Table 3.14 Glycerol 3-Phosphate Assay Kits

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

3.24 β-Hydroxybutyrate (β-HB) Assays

Ketone bodies are produced by liver and used peripherally as an energy source when blood glucose levels drop. The two main ketone bodies are β-hydroxybutyrate (β-HB) and acetoacetate (AcAc), while acetone is the third abundant ketone body. Normally these two predominant ketone bodies are present in small amounts in blood during fasting (low food intake) and prolonged exercise. In patients who have diabetes, alcohol or salicylate poisoning, hormone deficiency, childhood hypoglycemia and other acute disease states, large quantities of ketone bodies are found in blood. The over-production and accumulation of ketone bodies in blood (ketosis) can lead to pathological metabolic acidosis (ketoacidosis). In extreme cases, ketoacidosis can be fatal. Blood ketone testing methods that quantify β-HB, the predominant ketone body in blood (approximately 75%) have been used for diagnosing and monitoring treatment of ketoacidosis.

Amplite™ Colorimetric β-Hydroxybutyrate Assay Kit (Cat# 13830) offers a sensitive assay for measuring β-HB levels in biological samples. This assay is based on an enzyme coupled reaction of β-HB, in which the product NADH can be specifically monitored by a NADH sensor. The signal can be measured using an absorbance microplate reader with the OD ratio at the wavelength of 570 nm to 610 nm. With Amplite™ Colorimetric β-Hydroxybutyrate Assay Kit, as low as 4 μM β-HB was detected.

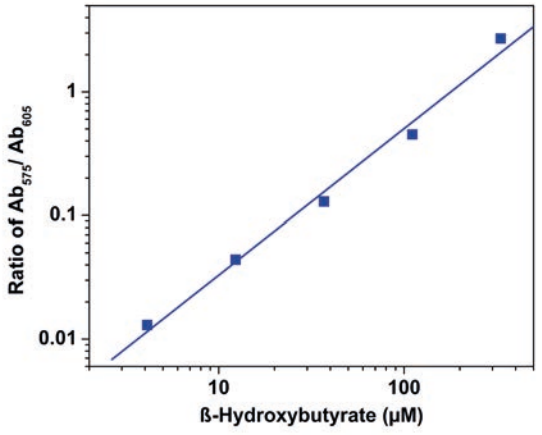

Figure 3.35. β-Hydroxybutyrate (β-HB) dose responses were measured with Amplite™ Colorimetric β-Hydroxybutyrate Assay Kit (Cat# 13830) on a 96-well black wall/clear bottom plate using a SpectraMax® microplate reader (Molecular Devices). As low as 4 μM β-HB was detected with 30 minutes incubation.

Amplite™ Fluorimetric β-Hydroxybutyrate Assay Kit (Cat# 13831) is also based on an enzyme coupled reaction of β-HB, in which the product NADH can be specifically monitored by a fluorescent NADH sensor. The fluorescence signal can be measured using a fluorescence microplate reader at Ex/Em= 540/590 nm. With Amplite™ Fluorimetric β-hydroxybutyrate Assay Kit, as low as 1.4 μM β-HB was detected.

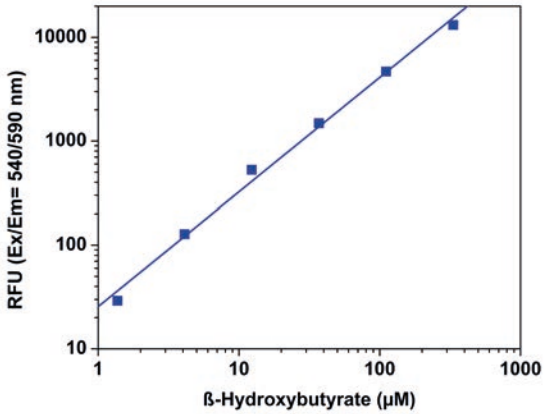

Figure 3.36. β-Hydroxybutyrate dose responses were measured with Amplite™ Fluorimetric β-Hydroxybutyrate Assay Kit (Cat# 13831) on a 96-well black solid plate. As low as 1.4 μM β-HB was detected with 10-30 minutes incubation.

Table 3.15 β-Hydroxybutyrate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13830	Amplite™ Colorimetric β-Hydroxybutyrate (Ketone Body) Assay Kit	200 tests	575	N/A
13831	Amplite™ Fluorimetric β-Hydroxybutyrate (Ketone Body) Assay Kit	200 tests	571	585

3.25 D-Lactate Assays

Lactic acid is chiral and has two optical isomers: L-lactic acid and D-lactic acid. Lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in the process of metabolism and exercise. Monitoring lactate levels is a good way to evaluate the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. D-lactic and L-lactic acids are found in many fermented milk products such as yoghurt and cheese, also in pickled vegetables, and in cured meats and fish. Abnormal high concentration of D-lactate in blood is usually a reflection of bacterial overgrowth in the gastrointestinal tract.

Amplite™ D-Lactate Assay Kits (Cat# 13810 and 13811) provide both fluorescence- and absorbance-based methods for detecting D-lactate in biological samples, such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, D-lactate is proportionally related to NADH, which is specifically monitored by a chromogenic or a fluorogenic NADH sensor. For Amplite™ Colorimetric D-Lactate Assay Kit (Cat# 13811), the signal can be easily read using an absorbance microplate reader at ~575 nm or at the absorbance ratio of ~575 nm/605 nm to increase assay sensitivity. With Amplite™ Colorimetric D-Lactate Assay Kit, as little as 4 μM D-lactate was detected.

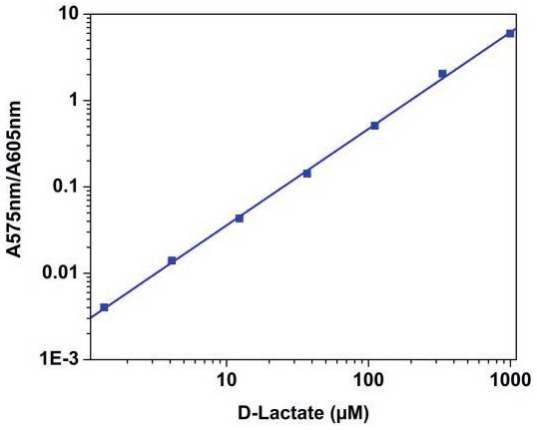

Figure 3.37. D-lactate dose responses were measured with Amplite™ Colorimetric D-Lactate Assay Kit (Cat# 13811) in a 96-well clear bottom plate. As low as 4 μM D-lactate was detected with 1 hour incubation.

For Amplite™ Fluorimetric D-Lactate Assay Kit (Cat# 13810), the signal is monitored by a fluorogenic NADH sensor. The signal can be read using a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With Amplite™ Fluorimetric D-Lactate Assay Kit, as little as 1.4 μM D-lactate was detected. The kit is robust, and can be readily adapted for a wide variety of applications that require the measurement of D-lactate.

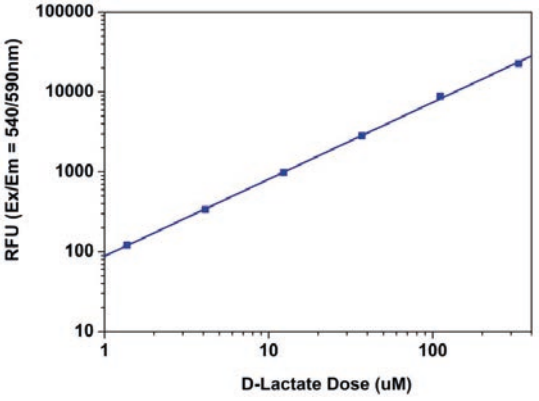

Figure 3.38. D-lactate dose responses were measured with Amplite™ Fluorimetric D-Lactate Assay Kit (Cat# 13810) in a 96-well black plate. As low as 1.4 μM D-lactate was detected with 1 hour incubation.

Table 3.16 D-Lactate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13811	Amplite™ Colorimetric D-Lactate Assay Kit	200 tests	575	N/A
13810	Amplite™ Fluorimetric D-Lactate Assay Kit	200 tests	571	585

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3.26 L-Lactate Assays

Amplite™ L-Lactate Assay Kits (Cat# 13814 & 13815) provide both fluorescence- and absorbance-based methods for detecting L-lactate in biological samples, such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, L-lactate is proportionally related to NADH, which is specifically monitored by a fluorogenic NADH sensor. With Amplite™ Fluorimetric L-Lactate Assay Kit (Cat# 13814), the signal can be read by a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. As little as 1.4 µM L-lactate was detected. With Amplite™ Colorimetric L-Lactate Assay Kit (Cat# 13815), the signal can be read by an absorbance microplate reader at ~575 nm or at the absorbance ratio of ~$A_{575\ nm}/A_{605\ nm}$ to increase assay sensitivity. As little as 4 µM L-lactate was detected.

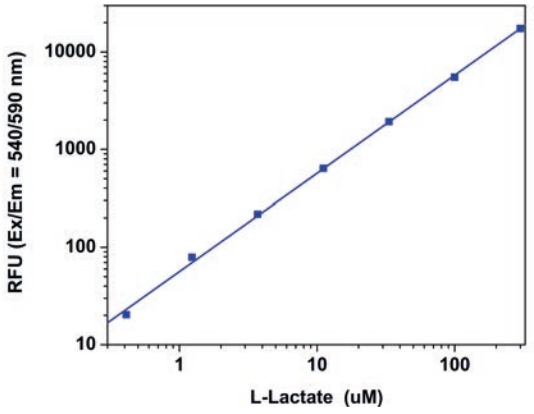

Figure 3.39. L-lactate dose responses were measured with Amplite™ Fluorimetric L-Lactate Assay Kit (Cat# 13814) in a 96-well black plate. As low as 1.4 µM L-lactatevolume was detected with 1 hour incubation.

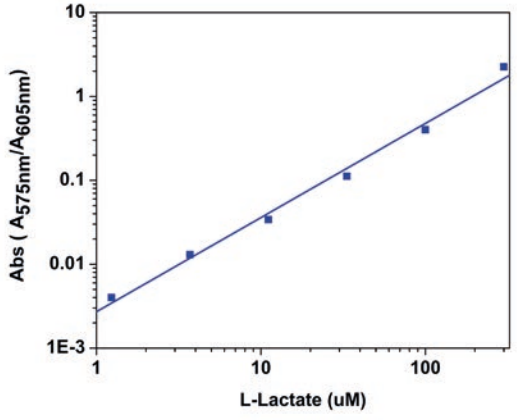

Figure 3.40. L-lactate dose responses were measured with Amplite™ Colorimetric L-Lactate Assay Kit (Cat# 13815) in a 96-well clear bottom plate. As low as 4 µM L-lactate was detected with 1 hour incubation.

Table 3.17 L-Lactate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13815	Amplite™ Colorimetric L-Lactate Assay	200 tests	575	N/A
13814	Amplite™ Fluorimetric L-Lactate Assay	200 tests	571	585

3.27 Lipid Droplet Assay

Lipid droplets, also referred to as lipid bodies, oil bodies or adiposomes, are lipid-rich cellular organelles that regulate the storage and hydrolysis of neutral lipids. They also serve as a reservoir of lipid source for many important biological processes such as fatty acid and cellular cholesterol for energy and membrane formation and maintenance. Abnormal accumulation of the cytoplasmic lipid droplets occurs in a variety of pathological conditions and can be an indicator of metabolic deficiency or pathogenesis.

Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit (Cat# 22730) is a simple assay that could quantitatively measure lipid droplet accumulation. Nile red (also known as Nile blue oxazone) is used in the kit for lipophilic stain. Nile red is intensely fluorescent in lipid-rich environment while it has minimal fluorescence in aqueous media. It is an excellent vital stain for the detection of intracellular lipid droplets with fluorescence microscopy, flow cytometry or microplate reader. Nile red stains intracellular lipid droplets red. The fluorescence signal could be read at Ex/Em = 485/550 nm or observed in FITC or TRITC channels. Better selectivity for cytoplasmic lipid droplets can be obtained when the cells are viewed for yellow-gold fluorescence (450-500 nm excitation; >528 nm emission) rather than red fluorescence (515-560 nm excitation; >590 nm emission).

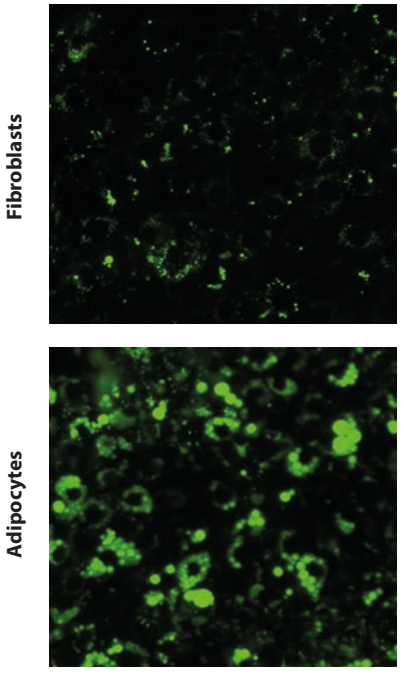

Figure 3.41. Images of 3T3-L1 cells stained with Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit (Cat# 22730) in a 96-well black solid plate.

Table 3.18 Lipid Droplet Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22730	Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit	200 tests	485	550

3.28 NAD/NADH & NADP/NADPH Detection

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

AAT Bioquest offers the most comprehensive product portfolio for NAD/NADH and NADP/NADPH detection as summarized in Table 3.19. All our NAD/NADH and NADP/NADPH assay kits are in a mix and read format with minimal hands-on time required. These kits have either significantly improved sensitivity and dynamic range or less interference from biological samples compared to the commercial assay kits from other vendors.

NAD Assays

AAT Bioquest has recently developed a set of fluorogenic probes that have excellent responses to NAD and NADP respectively. The new probes have enabled us to introduce the two newest NAD and NADP assay kits (Cat# 15280 & 15281). Amplite™ Fluorimetric NAD Assay Kit (Cat# 15280) provides a sensitive and rapid detection of NAD. The kit directly measures NAD using Quest Fluor™ NAD reagent, our newly developed NAD sensor. The proprietary probe reacts only with NAD to generate a product that fluoresces at Ex/Em = 420/480 nm, and has little response to NADH. As little as 30 nM NAD was detected, and 0.3% NAD generation was monitored in the presence of excess amount of NADH. This assay can be used in high-throughput screening.

Key Features of Amplite™ NAD/NADH & NADP/NADPH Assay Kits:

- ***Better Selectivity,*** minimal interference among NAD/NADH & NADP/NADPH.
- ***Broad Applications,*** quantify NAD/NADH & NADP/NADPH in a variety of media.
- ***Enhanced Sensitivity,*** detect as low as 30 nM NAD/NADH & NADP/NADPH.
- ***Great Convenience,*** minimal hands-on time. No wash is required.
- ***Multiple Modalities,*** absorption or fluorescence detection.
- ***Non-radioactive,*** no special requirements for waste treatment.

Table 3.19. NAD/NADH & NADP/NADPH Assay Comparison

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

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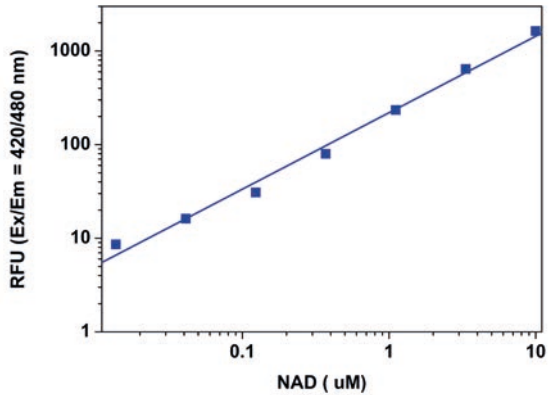

Figure 3.42. NAD dose responses were measured with Amplite™ Fluorimetric NAD Assay Kit (Cat# 15280) in a 96-well black solid plate. As low as 30 nM NAD was detected with 20 minute incubation.

NADH Assays

Amplite™ Colorimetric NADH Assay Kit (Cat# 15271) provides a convenient method for the detection of NADH. The NADH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NADH reduction. The absorbance increase at 460 nm is directly proportional to the concentration of NADH in the solution. Amplite™ Colorimetric NADH Assay Kit provides a sensitive assay to detect as little as 3 µM NADH. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

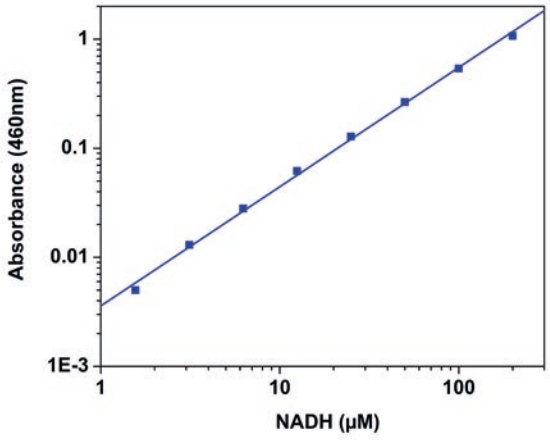

Figure 3.43. NADH dose responses were measured with Amplite™ Colorimetric NADH Assay Kit (Cat# 15271) in a 96-well clear bottom plate. As low as 3 µM NADH was detected with 30 minutes incubation.

Amplite™ Fluorimetric NADH Assay Kit (Cat# 15261) uses an enzyme coupled method for the detection of NADH. The enzymes in the system specifically recognize NADH in an enzyme recycling reaction. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference resulted from biological samples. Amplite™ Fluorimetric NADH Assay Kit provides a sensitive, one-step assay to detect as little as 100 picomoles NADH in a 100 µL assay volume (1 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read using either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm.

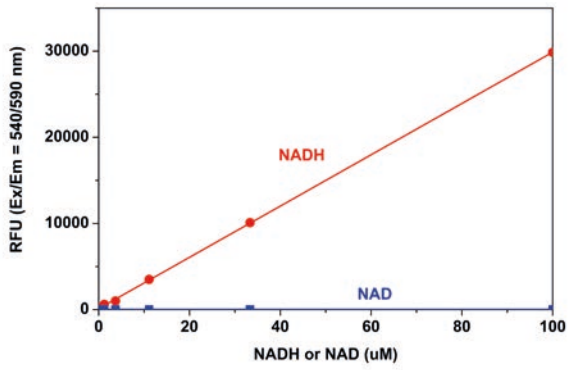

Figure 3.44. NADH dose responses were measured with Amplite™ Fluorimetric NADH Assay Kit (Cat# 15261) in a 96-well black solid plate. As low as 1 µM (100 pmol/well) NADH was detected with 1 hour incubation while there was no response from NAD.

Total NAD & NADH Assays

Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15258) provides a sensitive, one-step assay to detect as little as 30 picomoles of NAD/NADH in a 100 µL assay volume (300 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read using an absorbance microplate reader at ~575 nm or at the absorbance ratio of ~$A_{570\ nm}$ /$A_{605\ nm}$ to increase assay sensitivity.

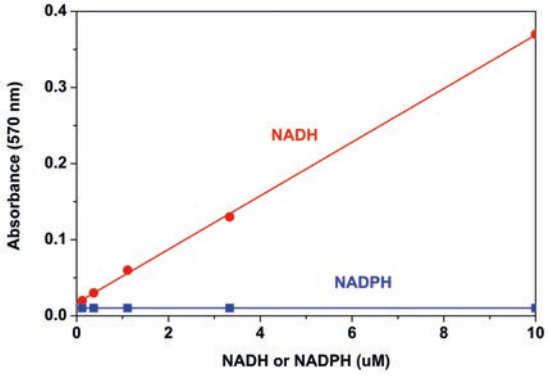

Figure 3.45. NADH dose responses were measured with Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15258) in a 96-well clear bottom plate. As low as 300 nM (30 pmol/well) NADH was detected with 1 hour incubation while there was no response from NADPH.

Through our continuous improvement and innovation, our new Amplite™ Colorimetric Total NAD and NADH Assay Kit 15275 achieved outstanding sensitivity. The enzymes in the system specifically recognize NAD and NADH in an enzyme cycling reaction, and thus significantly increase detection sensitivity. The NAD/NADH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NAD/NADH reduction. The absorption of the NAD/NADH probe is directly proportional to the concentration of NAD/NADH. Amplite™ Colorimetric Total NAD and NADH Assay Kit 15275 detects as little as 0.1 µM total NAD and NADH in a 100 µL assay volume. Compared to Amplite™ Colorimetric Total NAD and NADH Assay Kit 15258, Kit 15275 demonstrates higher sensitivity.

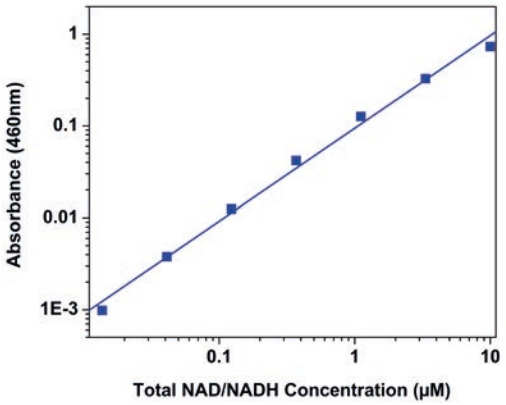

Figure 3.46. Total NAD and NADH dose responses were measured with Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15275) in a 96-well clear bottom plate. As low as 0.1 µM total NAD and NADH was detected with 1 hour incubation (n=3).

Amplite™ Fluorimetric Total NAD & NADH Assay Kit (Cat# 15257) provides a sensitive, one-step assay to detect as little as 10 picomoles NAD/NADH 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 readily adapted to automation without a separation step. Its signal can be easily read using either a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at ~576 nm.

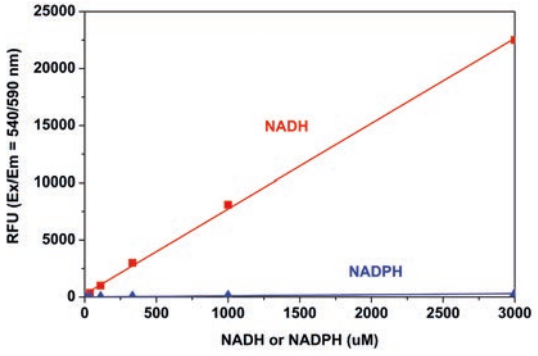

Figure 3.47. NADH dose responses were measured with Amplite™ Fluorimetric Total NAD and NADH Assay Kit (Cat# 15257) in a 96-well black solid plate. As low as 100 nM (10 pmol/well) NADH was detected with 1 hour incubation while there was no response from NADPH.

NAD/NADH Ratio Assays

NAD or NADP functions as a cofactor in redox reaction, transferring electrons in cellular reaction. The balance between the oxidized and reduced forms is NAD/NADH (NADP/NADPH) ratio. This ratio is an important component to indicate the redox state of a cell, and it is a measurement that reflects both the metabolic activities and the health of cells. In healthy mammalian tissues, the ratio between free NAD and NADH can be as high as 700. In contrast, the NADP/NADPH ratio is normally about 0.005, so NADPH is the dominant form of this coenzyme.

Amplite™ Colorimetric NAD/NADH Ratio Assay Kit (Cat# 15273) provides a colorimetric method for measuring intracellular total NAD and NADH amount and NAD/NADH ratio in culture cells. In the assay, NAD in the cell lysate can be extracted with NAD extraction solution and converted to NADH through enzyme reaction, and then recognized by the NADH probe to give a yellow-color dye after reaction, which has the absorbance at 460 nm. The amount of the dye generated is directly proportional to the concentration of NAD or NADH in the cell lysate and can be used as an indicator of the cellular NAD/NADH concentration.

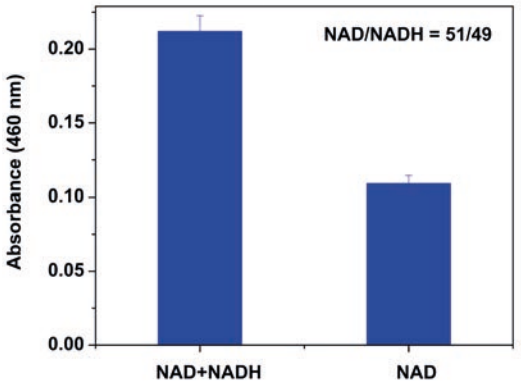

Figure 3.48. Amplite™ Colorimetric NAD/NADH Ratio Assay Kit (Cat#15273) was used to measure NAD/NADH ratio in a 96-well white wall/clear bottom microplate using a SpectraMax® microplate reader (Molecular Devices). Equal amount of NAD and NADH mixtures were treated with or without NAD extraction solution for 15 minutes, and then neutralized with extraction solution at room temperature. The signal was read at 460 nm. NAD/NADH ratio was calculated based on the absorbance shown in the figure.

Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit (Cat# 15263) provides a convenient method for sensitive detection of NAD, NADH and their ratio. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530 - 570 nm/590 - 600 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at ~576 nm. This kit provides NAD and NADH extraction buffer, and cell lysis buffer for your convenience. Kit 15263 has been frequently used for determining NAD/NADH from cell lysates.

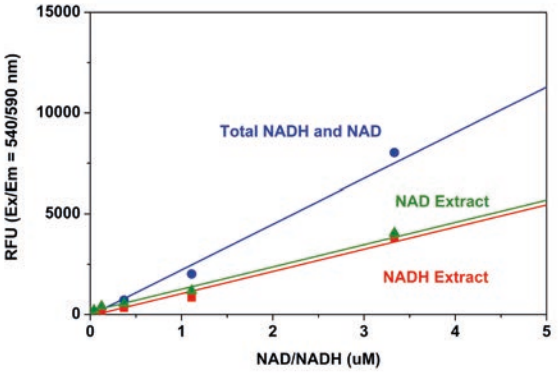

Figure 3.49. Total NAD and NADH, and their extract dose responses were measured with Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit (Cat# 15263) in a 96-well black plate. 25 µL of equal amount of NAD and NADH was treated with or without NADH or NAD extraction solution for 15 minutes, and then neutralized with extraction solutions at room temperature. The signal was acquired at Ex/Em = 540/590 nm (cut off at 570 nm) 30 minutes after adding 75 µL NADH reaction mixture. The blank signal was subtracted from the values of those wells with the NADH reactions

NADP Assays

Amplite™ Fluorimetric NADP Assay Kit (Cat# 15281) provides a sensitive and rapid detection of NADP. The kit directly measures NADP using Quest Fluor™ NADP reagent, our newly developed NADP sensor. The proprietary probe used in this kit reacts only with

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NADP to generate a product that fluoresces at Ex/Em = 420/480 nm, and has little response to NADPH. This kit can detect as little as 30 nM NADP, and monitor 0.3% NADP generation in the presence of excess amount of NADPH. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format and can be used in high-throughput screening.

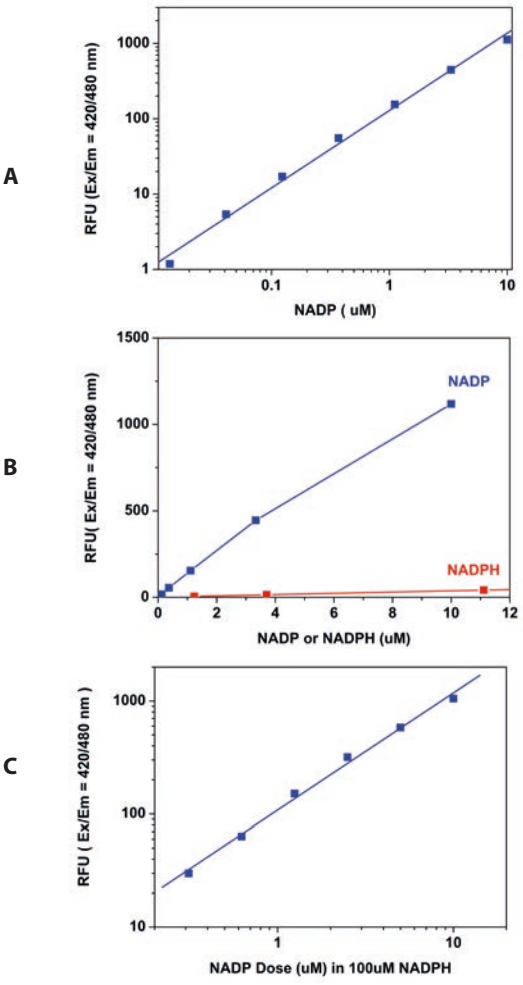

Figure 3.50. NADP dose responses were measured with Amplite™ Fluorimetric NADP Assay Kit (Cat# 15281) in a 96-well black solid plate. A: NADP standard curve, as low as 30 nM of NADP was detected with 20 minutes incubation (n=3). B: Comparison between NADP and NADPH responses. C: NADP standard curve in the presence of 100 µM NADPH in the solution. As low as 0.3% of NADP (~300 nM) converted from NADPH was detected with 20 minutes incubation.

NADPH Assays

Amplite™ Fluorimetric NADPH Assay Kit (Cat# 15262) provides a sensitive, one-step assay to detect as little as 100 picomoles of NADPH in a 100 µL assay volume (1 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. This assay kit has been used for the sensitive detection of NADPH in cell-based assays that use NADP/NADPH as a cofactor. Compared to the other commercial kits, Kit 15262 has higher signal/background ratio.

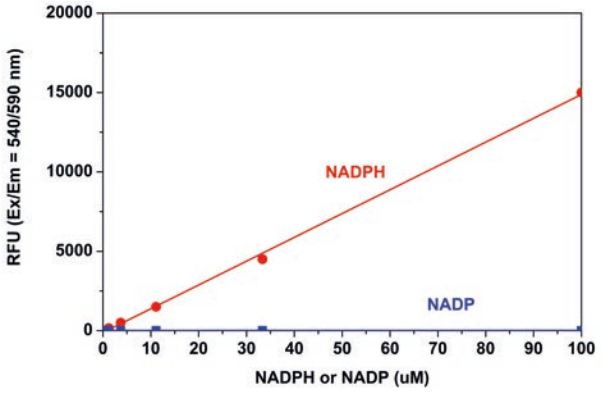

Figure 3.51. NADPH dose responses were measured with Amplite™ Fluorimetric NADPH Assay Kit (Cat# 15262) in a 96-well black solid plate. As low as 1µM NADPH was detected with 1 hour incubation while there was no response from NADP.

Amplite™ Colorimetric NADPH Assay Kit (Cat# 15272) provides a convenient method for detecting NADPH. The NADPH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NADPH reduction. The absorption of the NADPH probe is directly proportional to the concentration of NADPH in the solution. Amplite™ Colorimetric NADPH Assay Kit provides a sensitive assay to detect as little as 3 µM NADPH in a 100 µL assay volume.

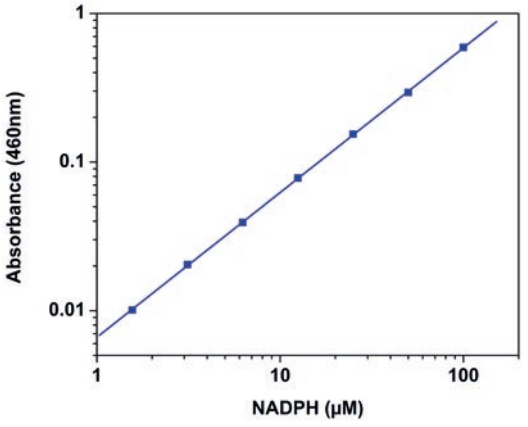

Figure 3.52. NADPH dose responses were measured with Amplite™ Colorimetric NADPH Assay Kit (Cat# 15272) in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular devices). As low as 3 µM NAPDH was detected with 30 minutes incubation (n=3).

Total NADP & NADPH Assays

Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat# 15260) provides a sensitive, one-step assay to detect as little as 10 picomoles of NADP(H) 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 readily adapted to automation without a separation step. Its signal can be easily read by an absorbance microplate

reader at ~575 nm or at the absorbance ratio of ~570 nm to ~605 nm to increase assay sensitivity. Kit 15259 or 15264 is recommended if higher sensitivity is required.

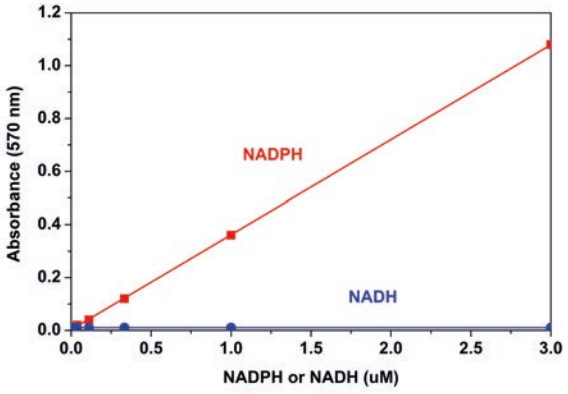

Figure 3.53. NADPH dose responses were measured with Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat# 15260) in a 96-well white wall/clear bottom plate using a NOVOStar microplate reader (BMG Labtech). As low as 100 nM (10 pmol/well) NADPH was detected with 1 hour incubation (n=3) while there was no response from NADH.

Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat# 15276) provides a convenient method for sensitive detection of NADP, NADPH and their ratio. The NADPH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NADH reduction. The absorption of the NADPH probe is directly proportional to the concentration of NADPH in the solution. Amplite™ Colorimetric Total NADP and NADPH Assay Kit provides a sensitive assay to detect as little as 0.03 µM total NADP and NADPH in a 100 µL assay volume. Compared to Amplite™ Colorimetric Total NADP and NADPH Assay Kit 15260, Kit 15276 demonstrates higher sensitivity.

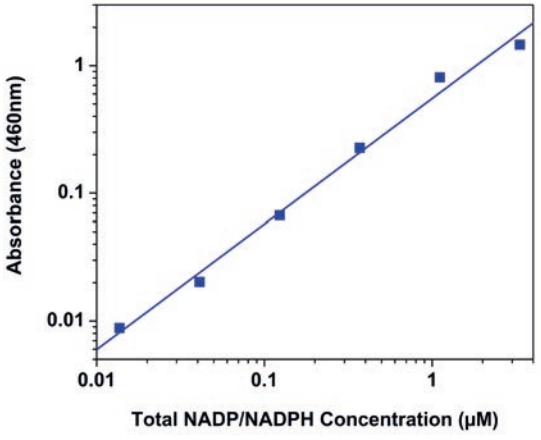

Figure 3.54. Total NADP and NADPH dose responses were measured with Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat# 15276) in a 96-well clear bottom plate. As low as 0.03 µM total NADP and NADPH was detected with 1 hour incubation (n=3).

Amplite™ Fluorimetric Total NADP and NADPH Assay Kit (Cat# 15259) provides a sensitive, one-step assay to detect as little as 1 picomoles of NADP(H) 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 readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. The longer red emission minimizes the interference from the autofluorescence of biological samples.

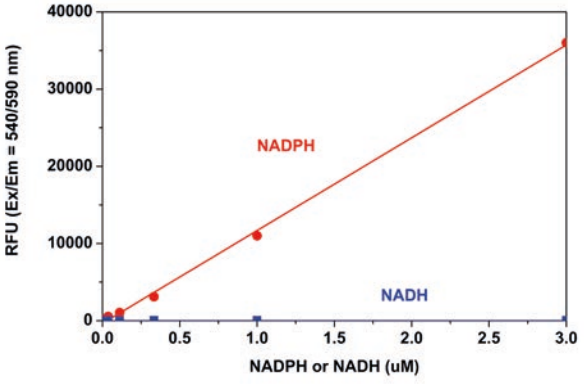

Figure 3.55. NADPH dose response was measured with Amplite™ Fluorimetric Total NADP and NADPH Assay Kit (Cat# 15259) in a 96-well black solid plate. As low as 10 nM (1 pmol/well) of NADPH was detected with 30 minutes incubation (n=3) while there was no response from NADH.

NADP/NADPH Ratio Assays

Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit (Cat# 15274) provides a colorimetric method for measuring intracellular total NADP and NADPH amount and NADP/NADPH ratio in cell culture. In the assay, NADPH in the cell lysate can be extracted with NADPH extraction solution and then recognized by the NADPH probe to give a yellow-color dye after reaction, which has the absorbance at 460 nm. The amount of the dye generated is directly proportional to the concentration of NADP or NADPH in the cell lysate and can be used as an indicator of the cellular NADP/NADPH concentration.

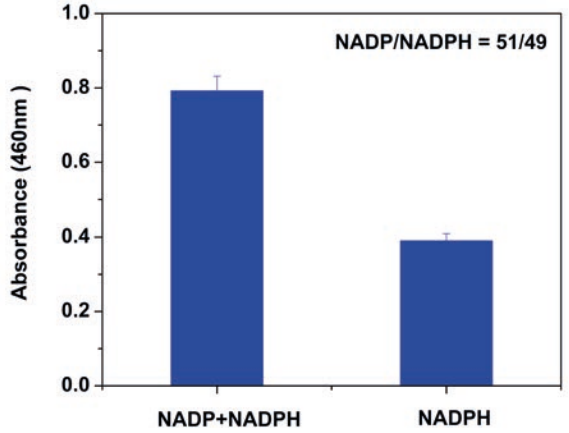

Figure 3.56. Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit (Cat# 15274) was used to measure NADP/NADPH ratio in a 96-well white wall/clear bottom microplate using a SpectraMax® microplate reader (Molecular Devices). Equal amount of NADP and NADPH mixtures were treated with or without NADPH extraction solution for 15 minutes, and then neutralized with extraction solution at room temperature. The signal was read at 460 nm. NADP/NADPH ratio was calculated based on the absorbance shown in the figure.

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Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit (Cat# 15264) provides a convenient method for sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme recycling reaction that significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that considerably reduces the sample interference. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530-570/590-600 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at ~576 nm. The kit also provides NADP, NADPH extraction buffer, and cell lysis buffer.

Table 3.20. NAD/NADH & NADP/NADPH Assay Kits

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

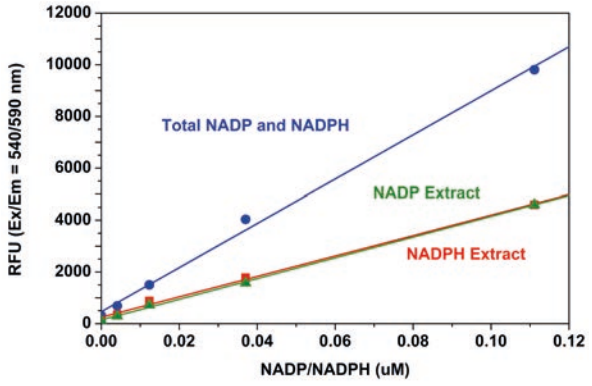

Figure 3.57. Total NADP and NADPH, and their extract dose responses were measured with Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit (Cat# 15264) in a 96-well black solid plate. The blank signal was subtracted from the values of those wells with the NADPH reactions.

3.29 Oxaloacetate Assays

Oxaloacetate is an important part of citric acid cycle, where it reacts with acetyl-CoA to form citrate. It is also involved in gluconeogenesis, urea cycle, glyoxylate cycle, amino acid synthesis, and fatty acid synthesis. The lack of oxaloacetate limits gluconeogenesis and urea cycle function, and can lead to decreased production of energy. And oxaloacetate can be also used as the blood glutamate scavengers to provide neuroprotection after traumatic brain injury, expressed both by reduced neuronal loss in the hippocampus and improved neurologic outcomes.

Amplite™ Oxaloacetate Assay Kits offer sensitive assays for quantifying oxaloacetate in biological samples. Oxaloacetate is converted to pyruvate and then utilizes an enzyme coupled reaction. The generated hydrogen peroxide can be detected by Amplite™ HRP substrate. The signal can be measured with either Amplite™ Colorimetric Oxaloacetate Assay Kit (Cat# 13840) using an absorbance microplate reader at 575 nm or Amplite™ Fluorimetric Oxaloacetate Assay Kit (Cat# 13841) using a fluorescence microplate reader at Ex/Em = 540/590 nm.

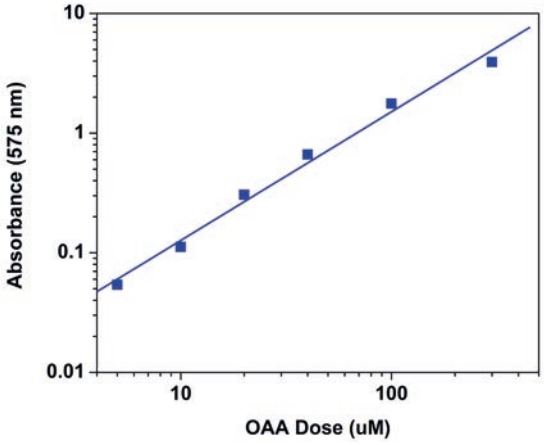

Figure 3.58. Oxaloacetate dose responses were measured with Amplite™ Colorimetric Oxaloacetate Assay Kit (Cat# 13840) on a 96-well clear bottom plate. As low as 5 µM oxaloacetate was detected with 30 minutes incubation.

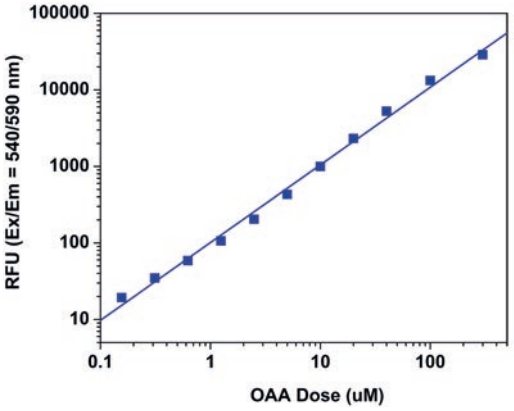

Figure 3.59. Oxaloacetate dose responses were measured with Amplite™ Fluorimetric Oxaloacetate Assay Kit (Cat# 13841) on a 96-well black solid plate. As low as 0.3 µM oxaloacetate was detected with 30 minutes incubation.

Table 3.21 Oxaloacetate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13840	Amplite™ Colorimetric Oxaloacetate Assay Kit *Red Color*	200 tests	575	N/A
13841	Amplite™ Fluorimetric Oxaloacetate Assay Kit *Red Fluorescence*	200 tests	571	585

3.30 Pyruvate Assays

Pyruvate is an important chemical compound in intracellular metabolic pathways. It is derived from metabolism of glucose known as glycolysis. One molecule of glucose breaks down into two molecules of pyruvate, which supplies living cells energy through one of two ways. When oxygen is present (aerobic respiration), pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase which enters citric acid cycles (also known as the Krebs cycle) to generate ATP. When oxygen is insufficient, pyruvate is broken down anaerobically, creating lactate in animals and ethanol in plants and microorganisms. Abnormal levels of pyruvate, or concentration ratio of lactate-to-pyruvate may be linked to liver disease or metabolic disorders. The detection of pyruvate levels is a diagnostic measurement in patient's clinical and other laboratory studies.

Amplite™ Colorimetric Pyruvate Assay Kit (Cat# 13821) offers a sensitive colorimetric assay for quantifying pyruvate in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by pyruvate sensor using an absorbance microplate reader at 575 nm.

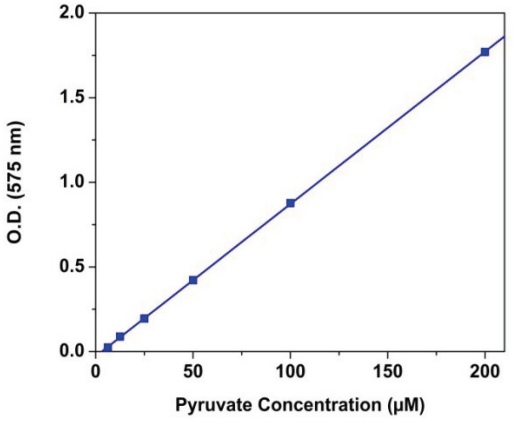

Figure 3.60. Pyruvate dose responses were measured with Amplite™ Colorimetric Pyruvate Assay Kit (Cat# 13821) on a 96-well clear bottom plate. As low as 6 µM pyruvate was detected with 30 minutes incubation.

Amplite™ Fluorimetric Pyruvate Assay Kit (Cat# 13820) offers a more sensitive fluorescent assay for quantifying pyruvate in biological samples. It also utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by pyruvate sensor using a fluorescence microplate reader at Ex/Em = 540/590 nm.

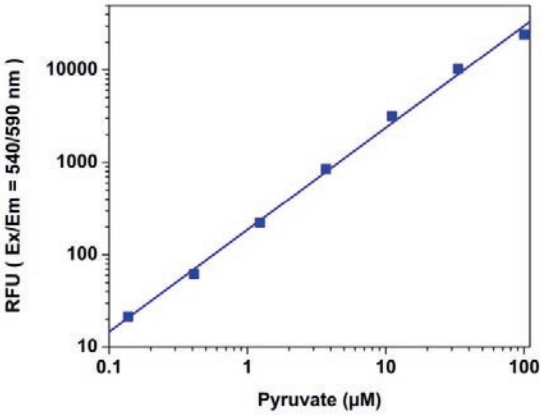

Figure 3.61. Pyruvate dose responses were measured with Amplite™ Fluorimetric Pyruvate Assay Kit (Cat# 13820) on a 96-well black solid plate. As low as 0.3 µM pyruvate was detected with 30 minutes incubation.

3.31 Sphingomyelin (SM) Assay

Sphingomyelin (SM) is largely found in the exoplasmic leaflet of the cell membrane, primarily in nervous tissue. It plays an important role in signal transduction. Sphingomyelin accumulates abnormally in Niemann-Pick disease and Abetalipoproteinemia.

Amplite™ Fluorimetric Sphingomyelin Assay Kit (Cat# 13625) provides the most sensitive method for detecting neutral SM activity or screening SM inhibitors. The kit uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). It can be used for measuring the amount of SM in blood, cell extracts or other solutions. The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the amount of SM. Amplite™ Red enables the

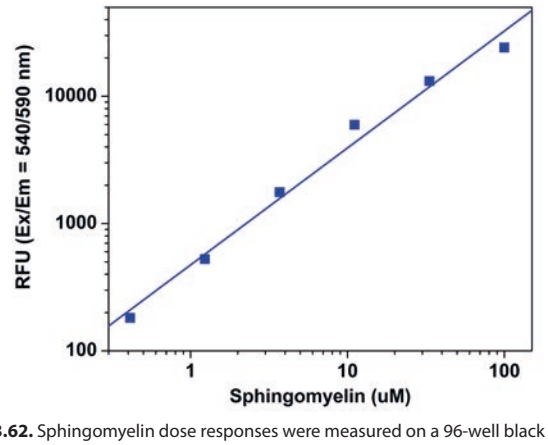

Figure 3.62. Sphingomyelin dose responses were measured on a 96-well black solid plate with Amplite™ Fluorimetric Sphingomyelin Assay Kit (Cat# 13625). As low as 1 µM sphingomyelin was detected with 60 minutes incubation.

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assay readable using either a fluorescence reader or an absorbance reader. The kit is an optimized "mix and read" assay that can be performed in a convenient 96-well or 384-well microtiter-plate format.

Table 3.22 Pyruvate & Sphingomyelin Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13821	Amplite™ Colorimetric Pyruvate Assay Kit	200 tests	575	N/A
13820	Amplite™ Fluorimetric Pyruvate Assay Kit	200 tests	571	585
13625	Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*	100 tests	571	585

3.32 Urea Assay

Urea is the final degradation product of protein and amino acid metabolism in animals. It is produced in liver, secreted by kidney and excreted through urine. The determination of urea is a very useful test in clinical laboratory studies to monitor health status. The Blood Urea Nitrogen (BUN) test is a measure of the amount of nitrogen in blood in the form of urea and is primarily used, along with the creatinine test, to evaluate kidney function, helping to diagnose kidney diseases.

Amplite™ Colorimetric Urea Quantitation Assay Kit (Cat# 10058) provides a simple and sensitive colorimetric method for the quantitation of urea concentration in biological samples such as serum, plasma and urine, etc. The assay is based on an enzyme-coupled reaction of urea in the assay buffer, and finally produces a blue colored product. The intensity of blue color produced is proportional to the concentration of urea in the sample, which can be measured colorimetrically at 660-670 nm. Amplite™ Colorimetric Urea Quantitation Assay Kit provides a simple assay to detect as little as 10 µM urea in a 150 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.

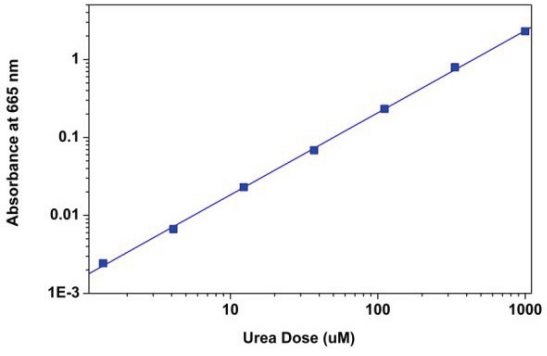

Figure 3.63. Urea dose responses were measured with Amplite™ Colorimetric Urea Quantitation Assay Kit (Cat# 10058) in a 96-well clear bottom plate using a SpectrumMax® microplate reader (Molecular Devices) . As low as 10 µM urea was detected in 15 minutes incubation after Assay Buffer II was added.

Table 3.23 Urea Quantitation Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
10058	Amplite™ Colorimetric Urea Quantitation Assay Kit *Blue Color*	200 tests	650	N/A

3.33 Xanthine Assays

Xanthine is a purine base found in most human body tissues and fluids. A number of stimulants are derived from xanthine, including caffeine, aminophylline, IBMX, paraxanthine, pentoxifylline, theobromine, and theophylline, which can stimulate heart rate, force of contraction, and cardiac arrhythmias at high concentrations. Therefore, detection of xanthine alteration in biological samples is important for disease diagnosis and therapy monitoring.

Amplite™ Xanthine Assay Kits provide quick and ultrasensitive methods for the measurement of xanthine. In the assay, xanthine is oxidized to uric acid by the action of xanthine oxidase. The product superoxide spontaneously degrades to hydrogen peroxide (H_2O_2), which can be specifically measured by Amplite™ Colorimetric Xanthine Assay Kit (Cat# 13842) using an absorbance microplate reader at 576±5 nm. Hydrogen peroxide can also be measured using Amplite™ Fluorimetric Xanthine Assay Kit (Cat# 13843) with a fluorescence microplate reader at Ex/Em = 530-570 nm/ 590-600 nm (optimal Ex/Em = 540 nm/590 nm).

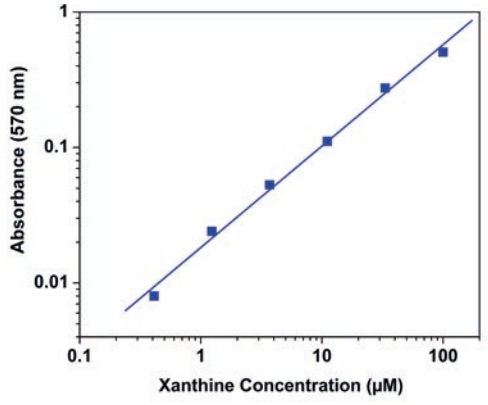

Figure 3.64. Xanthine dose responses were measured with Amplite™ Colorimetric Xanthine Assay Kit (Cat# 13842) in a 96-well clear bottom plate. As low as 1.2 µM xanthine was detected with 30 minutes incubation.

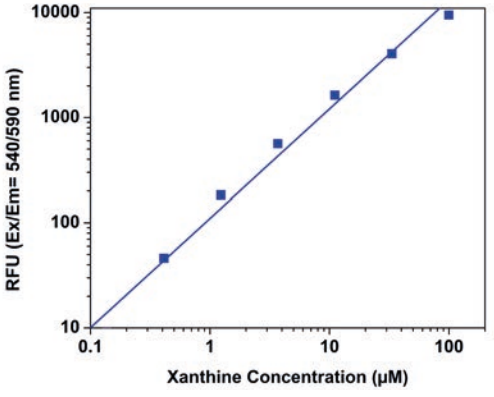

Figure 3.65. Xanthine dose responses were measured with Amplite™ Fluorimetric Xanthine Assay Kit (Cat# 13843) in a 96-well black solid plate. As low as 0.4 µM xanthine was detected with 30 minutes incubation.

Table 3.24 Xanthine Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13842	Amplite™ Colorimetric Xanthine Assay Kit	200 tests	571	N/A
13843	Amplite™ Fluorimetric Xanthine Assay Kit	200 tests	571	585

4 Detection of Reactive Oxygen Species (ROS)

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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.

4.1 Hydrogen Peroxide Assays & Probes

Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events that have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Perhaps the most intriguing aspect of H_2O_2 biology is the recent report that antibodies have the capacity to convert molecular oxygen into hydrogen peroxide to contribute to the normal recognition and destruction processes of the immune system. Measurement of this reactive species will help to determine how oxidative stress modulates varied intracellular pathways.

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501) uses our non-fluorescent Amplite™ Red peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. It provides a sensitive, one-step fluorimetric assay to detect as little as 3 picomoles of H_2O_2 in a 100 µL assay volume (30 nM). The assay can be performed in a convenient 96-well or 384-well microtiter plate format and readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~570 nm.

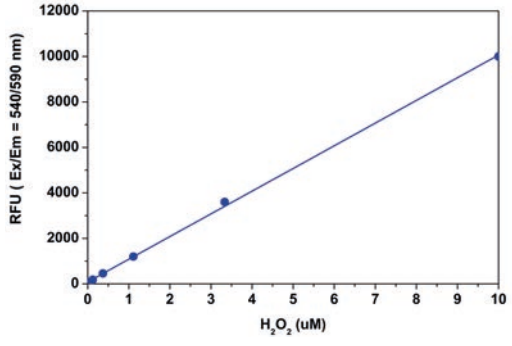

Figure 4.1. H_2O_2 dose responses were measured in a 384-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501). As low as 0.03 µM H_2O_2 was detected with 30 minutes incubation (n=3).

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502) uses our unique Amplite™ IR peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. Amplite™ IR generates the fluorescence that is pH-independent from pH 4 to 10. It is a superior alternative to ADHP (Amplex® Red) for the detections that require low pH where ADHP has reduced fluorescence. In addition, Amplite™ IR generates a product that has maximum absorption at 647 nm with maximum emission at 670 nm. This near infrared fluorescence minimizes the assay background that is often caused by the autofluorescence of biological samples. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions.

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit 11502 provides a sensitive, one-step fluorimetric assay to detect as little as 3 picomoles of H_2O_2 in a 100 µL assay volume (30 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 either a fluorescence microplate reader at Ex/Em = ~640/680 nm or an absorbance microplate reader at ~650 nm. Due to its long emission wavelength, this kit has low interference from biological samples.

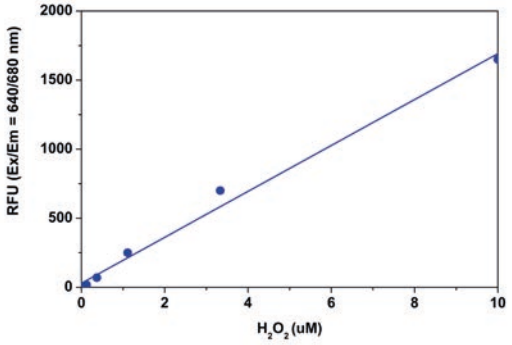

Figure 4.2. H_2O_2 dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502). As low as 0.03 µM H_2O_2 was detected.

Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503) uses our unique ROS Green™ to quantify hydrogen peroxide in live cells. ROS Green™ is cell-permeable, and generates the green fluorescence when it reacts with hydrogen peroxide. The kit is an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit provides a sensitive, one-

Table 4.1 Hydrogen Peroxide Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	571	585
11503	Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*	200 tests	492	515

step fluorimetric assay to detect as little as 0.3 nanomoles of H_2O_2 in a 100 µL assay volume (3 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 490/520 nm for H_2O_2 detection in solution or a fluorescence microscopy for live cell H_2O_2 imaging.

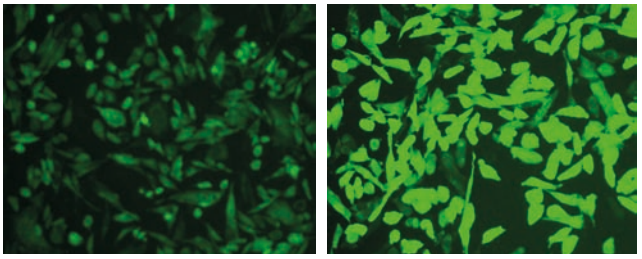

Figure 4.3. Images of live CHO-K1 cells in a 96-well plate. Live CHO-K1 cells were stained with Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503). Left: Control cells. Right: Cells treated with 100 µM H_2O_2 at room temperature for 5 minutes.

Dihydrofluorescein diacetate (also called fluorescin diacetate, Cat# 15203) is hydrolyzed by cellular esterases to dihydrofluorescein (also called fluorescin) and is then oxidized to fluorescein primarily by H_2O_2. Dihydrofluorescein diacetate might be reactive toward a broad range of oxidizing reactions that may be increased during intracellular oxidant stress. Cell-loading studies indicate that dihydrofluorescein diacetate achieves higher intracellular concentrations than the other redox sensors such as 2',7'-dichlorodihydrofluorescein and dihydrorhodamine 123.

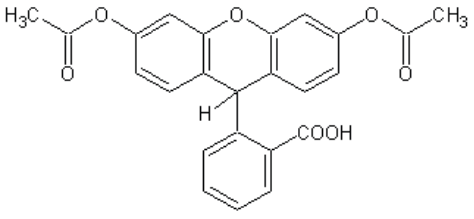

Figure 4.4. The chemical structure of dihydrofluorescein diacetate (Cat# 15203).

2',7'-Dichlorodihydrofluorescein diacetate (also called 2',7'-dichlorofluorescin diacetate, Cat# 15204) is hydrolyzed by cellular esterases to 2',7'-dichlorodihydrofluorescein (also called 2',7'-dichlorofluorescin) and is then oxidized to 2',7'-dichlorofluorescein primarily by H_2O_2. 2',7'-Dichlorodihydrofluorescein diacetate might be reactive toward a broad range of oxidizing reactions that may be increased during intracellular oxidant stress. This probe is widely used to monitor cellular redox processes. Compared to dihydrofluorescein diacetate, 2',7'-dichlorodihydrofluorescein diacetate-based H_2O_2 detection is less sensitive to pH.

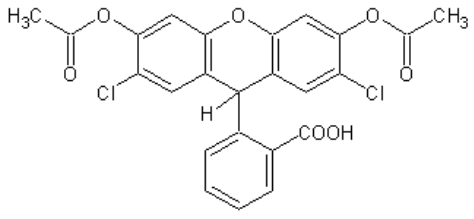

Figure 4.5. The chemical structure of 2',7'-dichlorodihydrofluorescein diacetate (Cat# 15204).

ROS Brite™ DHCF (Cat# 16053) has similar redox properties to those of 2',7'-dichlorodihydrofluorescein diacetate with significantly red-shifted spectra. ROS Brite™ DHCF is hydrolyzed by cellular esterases to generate the non-fluorescent reduced form that is then oxidized to generate the highly fluorescent free dye primarily by H_2O_2. ROS Brite™ DHCF might be reactive toward a broad range of oxidizing reactions that may be increased during intracellular oxidant stress. This probe can be conveniently used to monitor cellular redox processes for multiplexing assays with FITC-labeled antibodies or GFP cell lines. The oxidized product is highly fluorescent in cells. ROS Brite™ DHCF provides a valuable tool for investigating oxidative stress in various pathologies.

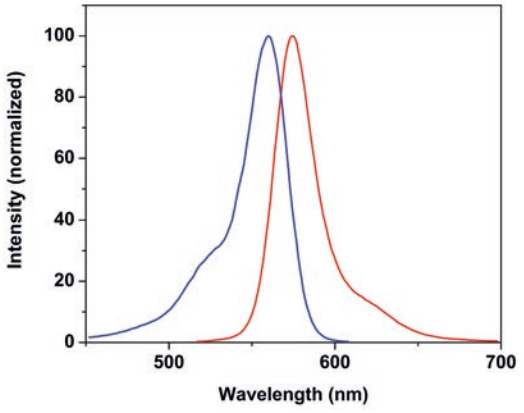

Figure 4.6. The excitation and emission spectra of ROS Brite™ DHCF (Cat# 16053) upon ROS oxidation in cells.

Table 4.2 Hydrogen Peroxide Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
15203	Dihydrofluorescein Diacetate [Fluorescin Diacetate]	25 mg	490	514
15204	2',7'-Dichlorodihydrofluorescein Diacetate [2',7'-Dichlorofluorescin Diacetate]	25 mg	504	529
16053	ROS Brite™ DHCF	1 mg	560	574

4.2 Hydroxyl Radical Probes

The cell-permeant ROS Brite™ APF (Cat# 16050) and HPF (Cat# 16051) reagents are nonfluorescent and produce bright green fluorescence upon reaction with hydroxyl radical. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. In the presence of peroxidase, APF and HPF also react with hydrogen peroxide. APF and HPF have good selectivity to hydroxyl radical compared to other ROS. They show relatively high resistance to light-induced oxidation. APF and HPF are nonfluorescent until they react with the hydroxyl radical or peroxynitrite anion. They might also react with the hypochlorite anion.

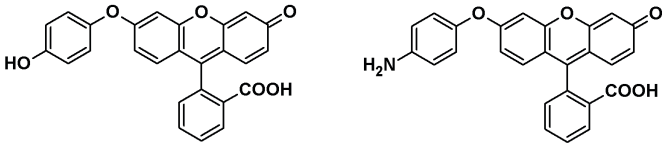

Figure 4.7. The chemical structures of ROS Brite™ APF (left, Cat# 16050) and HPF (right, Cat# 16051).

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Table 4.3 Hydroxyl Radical Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
16050	ROS Brite™ APF	1 mg	492	515
16051	ROS Brite™ HPF	1 mg	492	515

4.3 Hydroxyl Radical and Superoxide Probes

ROS Brite™ reagents are a series of new fluorogenic probes to measure oxidative stress in cells. The cell-permeant ROS Brite™ reagents are nonfluorescent and produce bright fluorescence upon ROS oxidation. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. ROS Brite™ 570, 670 and 700 reagents have good selectivity to both hydroxyl radical and supperoxide.

ROS Brite™ 570 (Cat# 16000) is a new fluorogenic probe to measure oxidative stress in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant ROS Brite™ 570 reagent is nonfluorescent and produces bright orange fluorescence upon ROS oxidation.

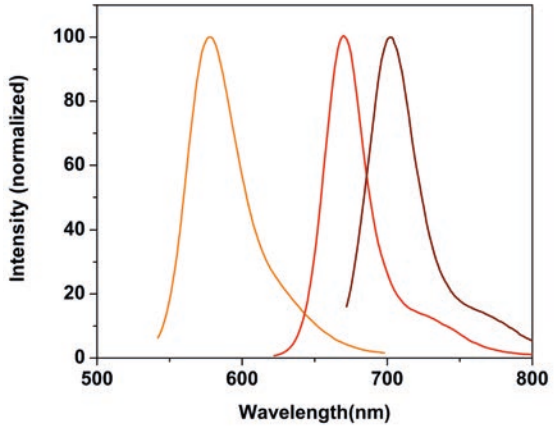

Figure 4.8. The fluorescence spectra of ROS Brite™ 570 (Yellow, Cat# 16000), ROS Brite™ 670 (Orange, Cat# 16002) and ROS Brite™ 700 (Red, Cat# 16004) in PBS buffer (pH 7.2).

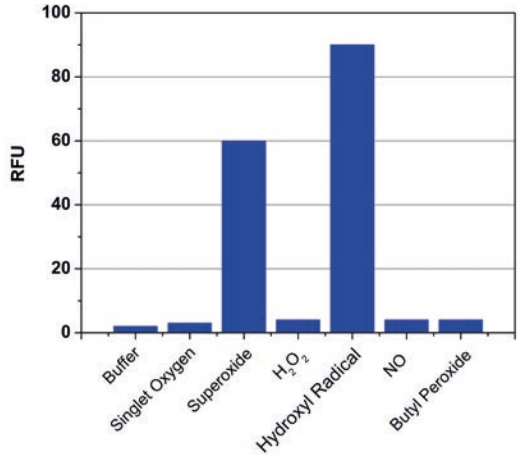

Figure 4.9. The responses of ROS Brite™ 570 (Cat# 16000) to different ROS species.

ROS Brite™ 670 (Cat# 16002) can be well excited with He-Ne laser at 633 nm, making this reagent well suited for the ROS detection using a flow cytometer. Its fluorescence signal can be well monitored using the Cy5® filter set.

ROS Brite™ 700 (Cat# 16004) is a new fluorogenic probe to measure oxidative stress in small animals. The cell-impermeant ROS Brite™ 700 reagent is water-soluble nonfluorescent and produces bright near-infrared fluorescence upon ROS oxidation. The resulting fluorescence can be measured using *in vivo* fluorescence imaging.

Table 4.4. Hydroxyl Radical and Superoxide Probes

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
16000	ROS Brite™ 570	1 mg	556	566
16002	ROS Brite™ 670	1 mg	658	675
16004	ROS Brite™ 700 *Optimized for *in vivo* Imaging*	1 mg	680	706

4.4 Nitric Oxide (NO) Probes

NO free radical is an important cellular signaling molecule involved in many physiological and pathological processes. It is an important biological regulator and is therefore a fundamental component in the fields of neuroscience, physiology, and immunology. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals such as nitroglycerine and amyl nitrite were discovered, more than a century after their first use in medicine, to be active through the mechanism of being precursors to nitric oxide. Low levels of nitric oxide production are important in protecting organs, such as the liver, from ischemic damage.

Key Features of DAX-J2™ NO Detection Probes:

- *No esterase activity required for NO detection.*
- *pH-independent spectral properties.*
- *Much more photostable than DAF-2.*
- *More tolerant to cell medium hydrolysis than DAF-2.*
- *Compatible with GFP cell lines or the applications that use FITC labeled antibodies for multicolor cell analysis.*

DAF-2 reagents are frequently used to detect nitric oxide (NO). However, DAF-2 diacetate is spontaneously hydrolyzed in cell culture media. The hydrolyzed DAF-2 is not cell-permeable, thus causing high assay background. DAX-J2™ probes are developed as excellent replacements for DAF-2 for the detection and bioimaging of NO. Compared to DAF-2 reagents, DAX-J2™ reagents have longer wavelengths and better stability. AAT Bioquest offers three distinct DAX-J2™ multicolor imaging reagents for NO detection.

DAX-J2™ Red (Cat# 16301) is a non-fluorescent cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells under physiological conditions. Once inside

the cell, the blocking groups on the DAX-J2™ reagent are released to generate a highly red fluorescent product upon NO oxidation. The DAX-J2™ fluorescent product can be detected using most flow cytometers and fluorescence microscopes equipped with the filter set of Texas Red®.

DAX-J2™ Orange (Cat# 16300) generates a bright orange fluorescent product that has spectra properties similar to those of Cy3® and TRITC. DAX-J2™ Orange can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy3® and TRITC.

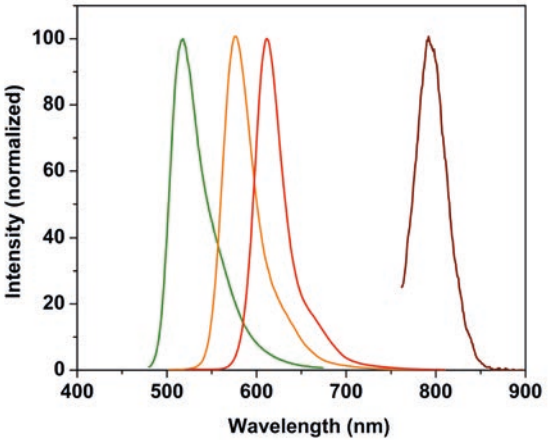

Figure 4.10. The spectral properties of DAX-J2™ reagents. DAF-2 (Green), DAX-J2™ Orange (Orange, Cat# 16300), Red (Red, Cat# 16301) and IR (Dark Red, Cat# 16302) in PBS buffer (pH 7.2).

DAX-J2™ IR (Cat# 16302) is a new fluorogenic NO sensor that has near infrared fluorescence. This DAX-J2™ IR reagent is highly water-soluble. It enables NO detection *in vivo* using IVIS® Imaging System(PerkinElmer) or Kodak Image Station.

DAX-J2™ Ratio 580/460 (Cat# 16310) is a new nitric oxide (NO) sensor recently developed by AAT Bioquest. It is a cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells in a ratiometric mode. Once inside the cell, the blocking groups on the DAX-J2™ reagent are released to induce fluorescence ratio changes at wavelengths of 580 nm and 460 nm upon NO oxidation. The fluorescence intensities at 580 nm and 460 nm can be detected using the filter sets of Cy3®/TRITC and BD Horizon™ V450/Pacific Blue. Most of flow cytometers and fluorescence microscopes are equipped with these two filter sets. DAX-J2™ Ratio 580/460 has distinct advantages for NO detection over the popular DAF-2 NO probe: 1). DAX-J2™ Ratio 580/460 does not require esterase activity for NO detection. DAF-2 requires intracellular esterases to cleave its acetate groups for detecting NO activity. 2). DAX-J2™ product exhibits pH-independent fluorescence while DAF-2 has its fluorescence highly affected by pH. 3). DAX-J2™ Ratio 580/460 can be monitored in a ratiometric mode.

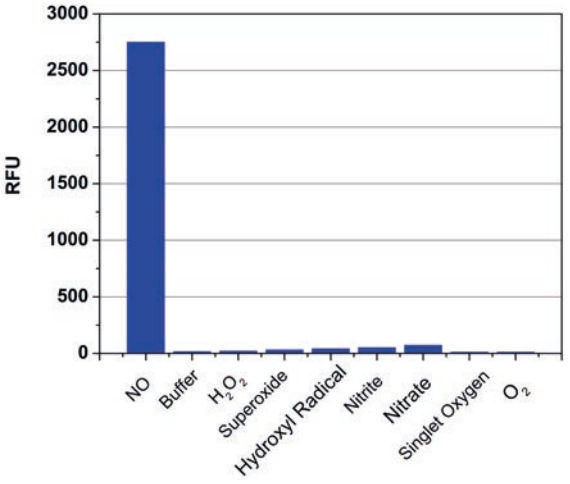

Figure 4.11. Fluorescence responses of DAX-J2™ Orange (5 µM, Cat# 16300) to different reactive oxygen species (1 mM) in PBS buffer (pH 7.2). The fluorescence intensities were measured at Ex/Em = 540/570 nm.

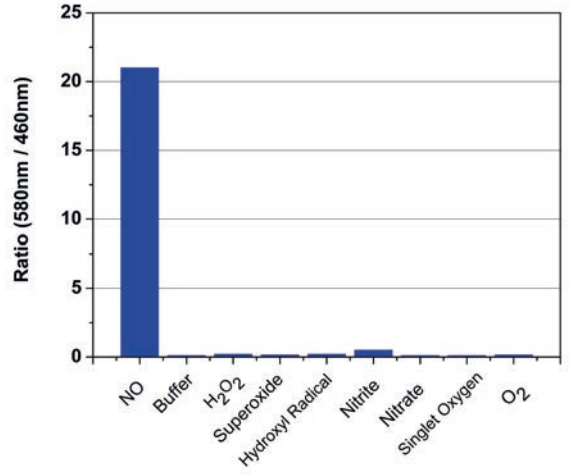

Figure 4.12. Fluorescence responses of DAX-J2™ Ratio 580/460 (2 µM, Cat# 16310) to different reactive oxygen species (1 mM) in PBS buffer (pH = 7.2). The fluorescence intensities were measured at 580 nm and 460 nm respectively.

Table 4.5. Multicolor Nitric Oxide (NO) Probes

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
16302	DAX-J2™ IR	1 mg	780	800
16300	DAX-J2™ Orange	1 mg	545	576
16310	DAX-J2™ Ratio 580/460	1 mg	420/540	460/580
16301	DAX-J2™ Red	1 mg	588	610

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4.5 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.

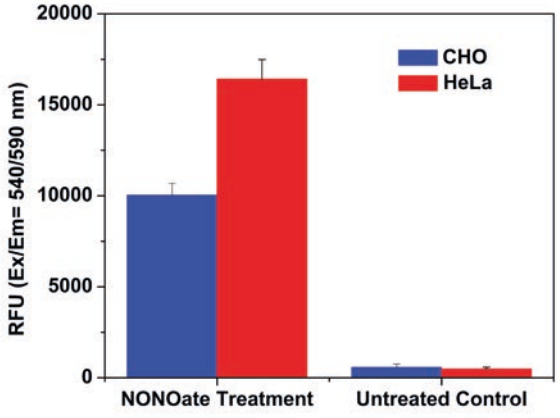

Figure 4.13. Detection of exogenous nitric oxide (NO) in cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16350). CHO-K1 and HeLa cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were incubated with Nitrixyte™ Orange working solution at 37 ºC for 30 minutes. The cells were treated with or without 1mM DEA NONOate at 37 ºC for 30 minutes. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode.

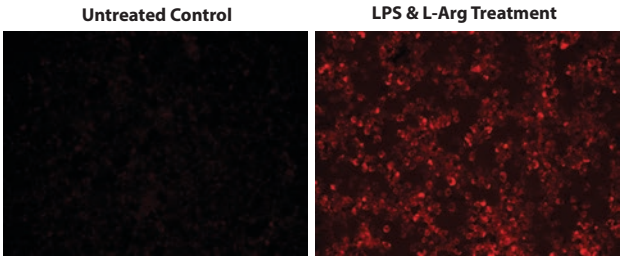

Figure 4.14. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16350). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells 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 ºC for 16 hours. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

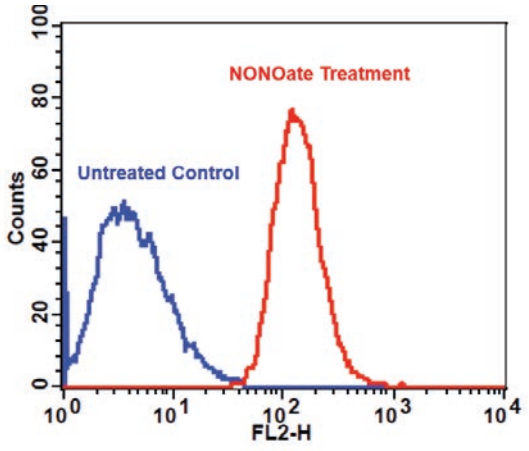

Figure 4.15. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16351). Cells were incubated with Nitrixyte™ Orange at 37 ºC for 30 minutes and washed twice with assay buffer. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 ºC for 30 minutes. The fluorescence signal was monitored in FL2 channel.

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®. Kit 16356 is optimized for flow cytometry applications.

Table 4.6 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

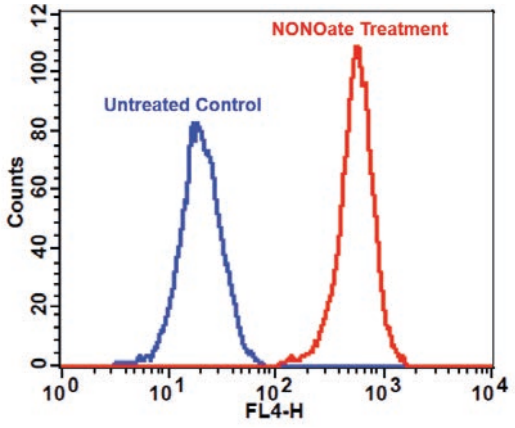

Figure 4.16. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat# 16356). Cells were incubated with Nitrixyte™ Red at 37 °C for 30 minutes. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 2 hours. The fluorescence signal was monitored using a flow cytometer (BD FACSCalibur™) in FL4 channel.

4.6 Intracellular Total ROS Activity Assays

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 & 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).

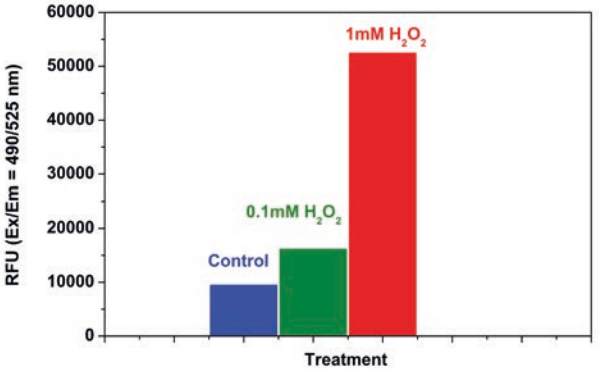

Figure 4.17. Detection of ROS in Jurkat cells using Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22900). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. And then the cells were treated with 1 mM, 0.1 mM or without H_2O_2 for 30 minutes.

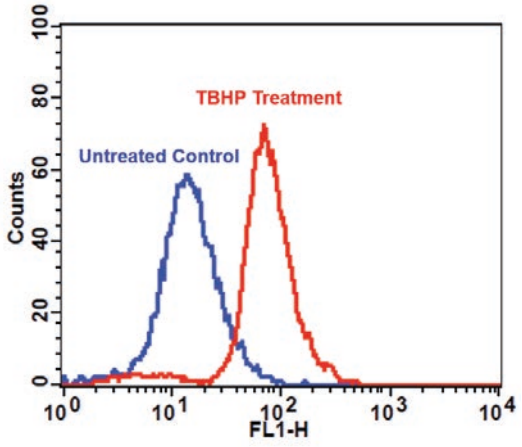

Figure 4.18. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22904). Cells were incubated with Amplite™ ROS Green at 37 °C for 1 hour. Cells were then treated with (Red) or without (Blue) 100 µM TBHP at 37 °C for 30 minutes. The fluorescence signal was monitored using a flow cytometer (BD FACSCalibur™) in FL1 channel.

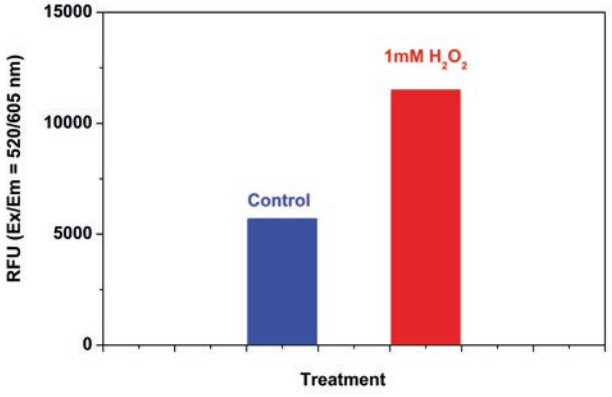

Figure 4.19. Detection of ROS in Jurkat cells using Amplite™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22901). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was 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.

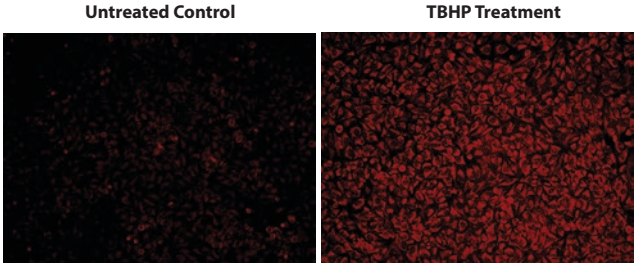

Figure 4.20. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22902) in a Costar 96-well black wall/clear bottom plate. Left: Untreated control cells. Right: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

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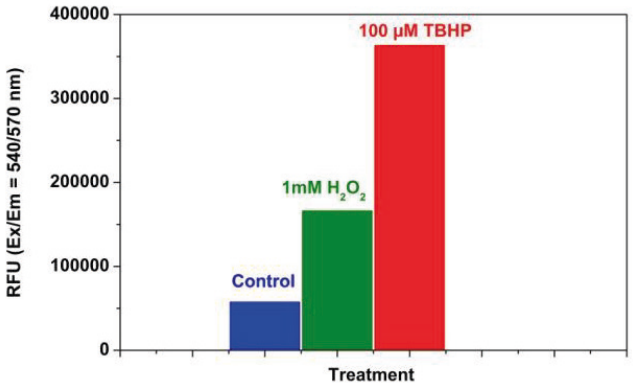

Figure 4.21. Detection of ROS in HeLa cells. HeLa cells were seeded overnight at 15,000 cells/90 µL/well in a Costar 96-well black wall/clear bottom plate. The cells were untreated (control) or treated with 1 mM H_2O_2 or 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. The ROS Brite™ 570 (Cat# 16000) (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).

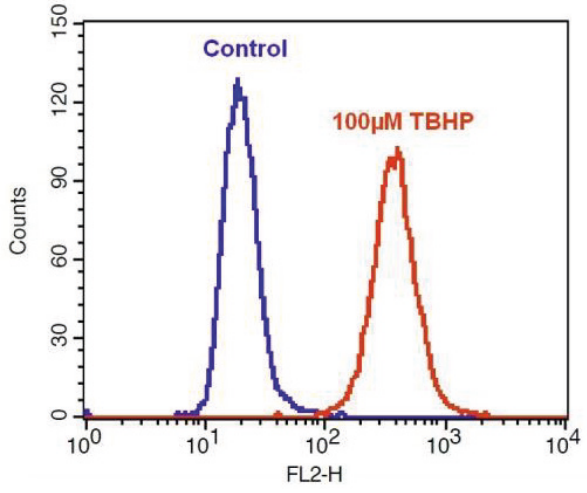

Figure 4.22. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 570 (Cat# 16000) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

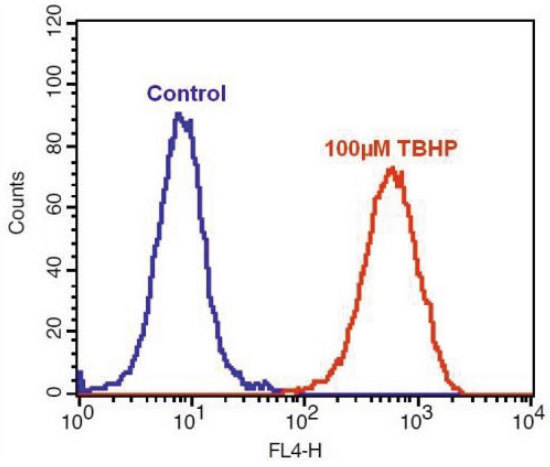

Figure 4.23. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 670 (Cat# 16002) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

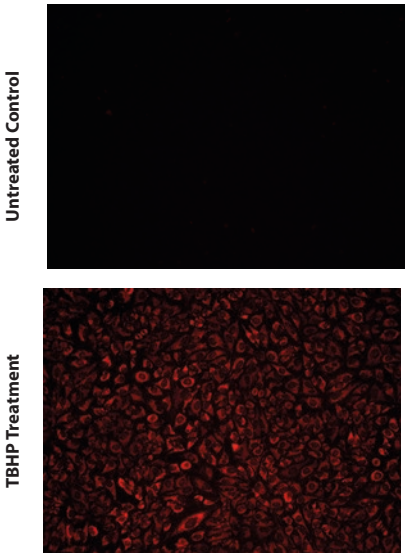

Figure 4.24. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22903) in a Costar 96-well black wall/clear bottom plate. Top: Untreated control cells. Bottom: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

Table 4.7 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	658	675
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
16000	ROS Brite™ 570	1 mg	556	566
16002	ROS Brite™ 670	1 mg	658	675

5 Quantification of Biopolymers

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5.1 dsDNA Quantification 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 5.1). This 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.

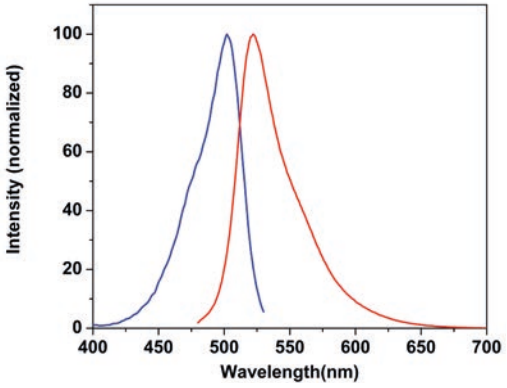

Figure 5.1. The excitation and emission spectra of Helixyte™ Green in the presence of calf thymus DNA.

Helixyte™ Green dsDNA Quantifying Reagent (Cat# 17597)can accurately quantify as little as 100 pg/mL dsDNA using a fluorometer or 300 pg/mL dsDNA (typically 50 pg in a 200 µL volume) using a fluorescence microplate reader. The Helixyte™ Green dsDNA quantitation 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 a >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. In comparison to the other common DNA dyes (such as Hoechst 33258 dye), which show significant base selectivity, Helixyte™ Green dsDNA Quantifying Reagent shows little if any AT- or GC-selectivity, enabling accurate DNA quantification.

The protocol of the Helixyte™ Green dsDNA quantitation assay is in a simple mix and read format, i.e., the dye is simply added to the sample and incubated for five minutes, then the fluorescence is measured. In addition, the fluorescence signal from binding of the Helixyte™ Green reagent to dsDNA is linear over at least four orders of magnitude with a single dye concentration, whereas assays using ethidium bromide or Hoechst 33258 dye exhibit a much more limited linear range. The 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. The Helixyte™ Green reagent can be excited at 488 nm with an argon-ion laser, and is a superior nucleic acid stain for analysis of single DNA molecules using a flow cytometer.

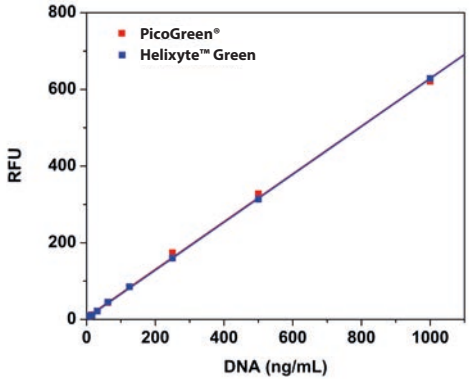

Figure 5.2. The quantification of calf thymus DNA with Helixyte™ Green vs. PicoGreen®. Helixyte™ Green and PicoGreen® have almost identical performance.

The Helixyte™ Green assay is useful for quantifying DNA templates for PCR, labeling reactions, electrophoretic mobility-shift (band-shift) assays, DNA-footprinting assays and filter-binding assays, and for measuring yields from PCR reactions, DNA minipreps and maxipreps, cDNA synthesis and nuclease protection assays. The simplicity and selectivity of the assay also make it ideal for high-throughput automated quantification assays used in forensic and genomics research.

Hoechst 33258 (Cat# 17520) has been extensively used to quantify dsDNA in solution. Hoechst 33258 shows a fluorescence increase upon binding nucleic acids and a preference for binding to AT regions. Hoechst 33258 is selective for dsDNA over RNA in high-salt buffers and for dsDNA over ssDNA in low-salt buffers. Hoechst 33258 can quantitatively detect from 10 ng/mL to ~10 µg/mL dsDNA when two different dye concentrations are used. While this assay uses principles that are similar to other fluorescent assays, newer dyes, such as the Helixyte™ Green reagent, provide much higher sensitivity, better selectivity and a broader dynamic range with a single dye concentration.

Table 5.1 dsDNA Quantification Reagents and Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17597	Helixyte™ Green dsDNA Quantifying Reagent	1 mL	501	520
17598	Helixyte™ Green dsDNA Quantifying Reagent	10 mL	501	520
17650	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *Optimized for Microplate Readers*	200 tests	501	520
17651	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit	200 tests	501	520
17520	Hoechst 33258 *UltraPure Grade*	100 mg	352	461

5.2 RNA Quantification Assays

StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17655) 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 5.3). 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 agaroses. 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.

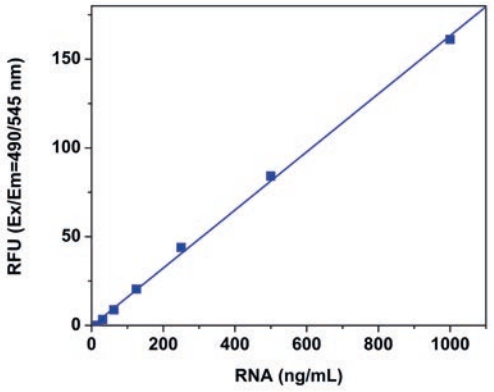

Figure 5.3. The quantification of RNA with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17655).

StrandBrite™ Green RNA Quantitation Reagent (Cat# 17611) allows detection of as little as 10 ng/mL RNA using a standard fluorometer, fluorescence microplate reader or filter-based fluorometer with standard fluorescein excitation and emission settings (Figure 5.4). 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 Quantitation Reagent does not detect significant sample contamination caused by free nucleotides. Thus, the StrandBrite™ Green RNA Quantitation Reagent more accurately measures the amount of intact RNA polymers in potentially degraded samples.

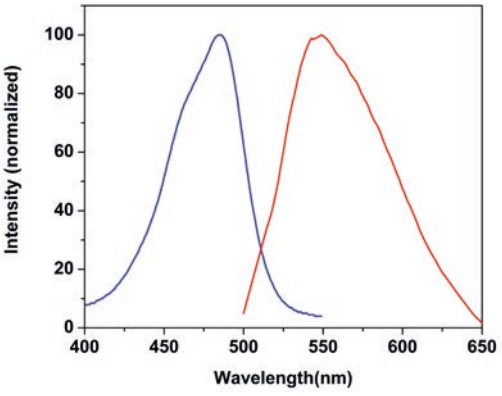

Figure 5.4. The excitation and emission spectra of StrandBrite™ Green in the presence of 1 ng/mL RNA.

StrandBrite™ Green RNA Quantitation Reagent is not appreciably selective for RNA. It also shows significant fluorescence enhancement upon binding to DNA. This interference might be overcome by a simple DNase pretreatment of samples to remove the contribution of DNA to the signal. The StrandBrite™ Green reagent may also have some base selectivity, thus may potentially affect its accuracy in quantifying homopolymers, e.g., poly(G), poly(U), or poly(C) and poly(A). Using the StrandBrite™ Green RNA Quantification Reagent, RNA was reproducibly quantified from a wide variety of sources, including ribosomal RNA (rRNA), transfer RNA (tRNA), viral RNA and total cellular RNA.

Table 5.2 RNA Quantification Reagent and Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17655	StrandBrite™ Green Fluorimetric RNA Quantification Kit *Optimized for Microplate Readers	200 tests	485	549
17610	StrandBrite™ Green RNA Quantifying Reagent	1 mL	485	549

5.3 Protein Quantification Assay

Protein quantification is necessary in protein purification, electrophoresis, cell biology, molecular biology, and other research applications. Biuret, Lowry, BCA and Bradford assays are routinely used for estimating protein concentration. However, these colorimetric assays are less sensitive, and require large sample volume to ensure higher accuracy. Our fluorescamine-based protein quantification kit is significantly more sensitive than existing standard colorimetric measurements, e.g., Bradford and Bicinchoninic acid (BCA) assays. Fluorescamine is intrinsically nonfluorescent but reacts rapidly with primary aliphatic amines, including those in peptides and proteins, to yield a blue-green-fluorescent derivative.

Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit (Cat# 11100) provides a simple method for quantifying protein concentration in solutions. As little as 3 µg/mL BSA was detected. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format. It can be completed within 30 minutes with the fluorescence signal easily monitored at Ex/Em = 380/470 nm. This kit has been used for (1). studying protein/protein interactions; (2). measuring column fractions after affinity chromatography; (3). estimating percent recovery of membrane proteins from cell extract; and (4). high-throughput screening of fusion protein.

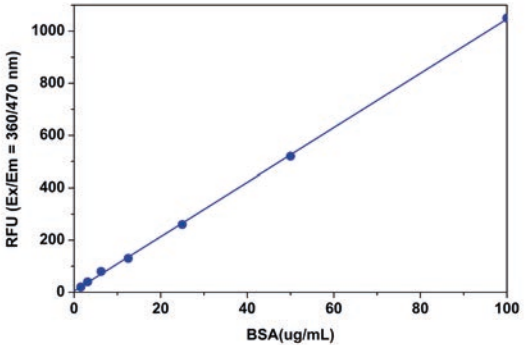

Figure 5.5. BSA dose responses were measured on a 96-well back plate with Amplite™ Fluorimetric Fluorescamine Protein Quantitation Assay Kit (Cat# 11100). As low as 3 µg/mL BSA was detected with 5 minutes incubation (n=3).

Table 5.3 Fluorescamine Protein Quantitation Assay Kit

Cat #	Product Name	Size	Ex (nm)	Em (nm)
11100	Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit *Blue Fluorescence*	200 tests	380	464

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Quantification of Ions 6

6.1 Calcium Quantification

Calcium is essential for all living organisms, particularly in cell physiology, where the movement of calcium ion into and out of the cytoplasm functions as a signal for many cellular processes. Calcium also plays an important role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. The serum level of calcium is closely regulated (9 to 10.5 mg/dL) in the human body. Both hypocalcemia and hypercalcemia are serious medical disorders.

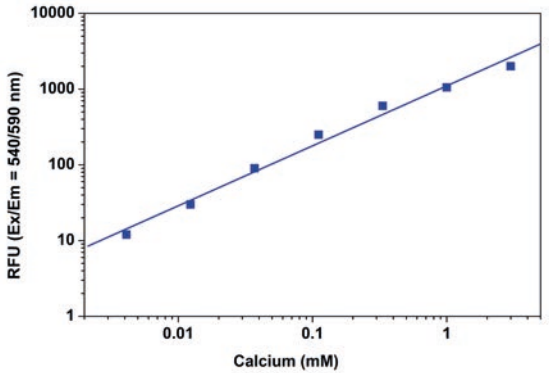

Figure 6.1. Calcium dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Calcium Quantitation Kit (Cat# 36360). As low as 0.03 mM calcium was detected with 5 minutes incubation (n=3).

Amplite™ Fluorimetric Calcium Quantitation Kit (Cat# 36360) provides a simple method for detecting calcium in physiology 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. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The assay can be completed within 30 minutes. With Amplite™ Fluorimetric Calcium Quantitation Kit, as little as 0.03 mM calcium was detected. The kit has a broad dynamic range (30 µM to 10 mM). If more sensitive calcium detection is required, we recommend that Fluo-8® or Fluo-3 be used instead. Both Fluo-8® and Fluo-3 can be used for determining calcium in nM range.

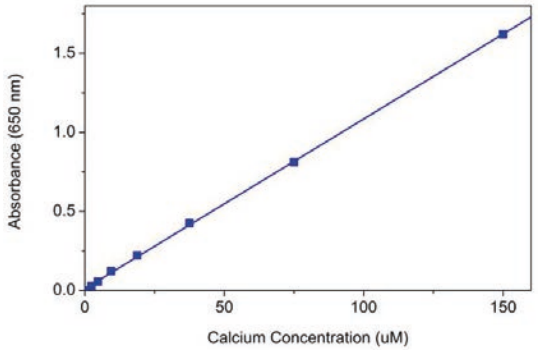

Figure 6.2. Calcium dose responses were measured in a 96-well black plate with Amplite™ Colorimetric Calcium Quantitation Kit (Cat# 36361). As low as ~ 2.5µM Ca^{2+} was detected with 5 minutes incubation (n=3).

Amplite™ Colorimetric Calcium Quantitation Kit (Cat# 36361) provides a simple method for detecting calcium in solutions. This kit uses our Calcium Blue™ as the chromogenic calcium indicator. Its absorbance changes in response to calcium binding. The absorbance signal can be easily read using an absorbance microplate reader at 600 nm or 650 nm. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format within 5 minutes and easily adapted to automation without a separation step. With Amplite™ Colorimetric Calcium Quantitation Kit, the calcium detection linear range is from 0.1 nmoles to 7.5 nmoles in 100 µL final test volume (2.5 to 150 µM calcium).

Table 6.1 Calcium Quantification Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
36361	Amplite™ Colorimetric Calcium Quantitation Kit *Blue Color*	200 tests	652	N/A
36360	Amplite™ Fluorimetric Calcium Quantitation Kit *Red Fluorescence*	200 tests	551	577

6.2 Chloride Detection

Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a cAMP-activated ATP-gated anion channel, increasing the conductance for certain anions (e.g. Cl^-) to flow down their electrochemical gradient. ATP-driven conformational changes in CFTR open and close a gate to allow transmembrane flow of anions down their electrochemical gradient. The measurement of intracellular chloride concentrations and the study of chloride channels have been stimulated by the discovery that cystic fibrosis is caused by mutations in a gene encoding CFTR. Chloride permeability assays are used to detect the activities of the CFTR and other anion transporters. A number of chronic disease states such as cystic fibrosis and Bartter's syndrome are due to defects in chloride channel functions.

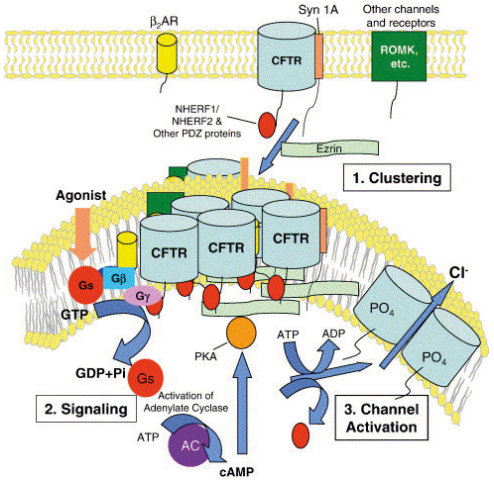

Figure 6.3. Cystic fibrosis transmembrane conductance regulator (CFTR) is a protein encoded by the CFTR gene. CFTR is an ABC transporter-class ion channel that transports chloride and thiocyanate ions across epithelial cell membranes. Mutations of the CFTR gene affect functioning of the chloride ion channels in these cell membranes, leading to cystic fibrosis and congenital absence of the vas deferens. The gene that encodes the CFTR protein is found on the human chromosome 7, on the long arm at position q31.2 from base pair 116,907,253 to base pair 117,095,955. CFTR orthologs have also been identified in all mammals for which complete genome data are available. The CFTR gene has been used in animals as a nuclear DNA phylogenetic marker. Large genomic sequences of this gene have been used to explore the phylogeny of the major groups of mammals, and confirmed the grouping of placental orders into four major clades: Xenarthra, Afrotheria, Laurasiatheria, and Euarchonta plus Glires.

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Quinolinium-Based Fluorescent Chloride Indicators

Most of the existing fluorescent chloride indicators are derived from quinolinium, including MEQ, MQAE and SPQ. All of these indicators detect chloride via diffusion-limited collisional quenching. This detection mechanism is different from that of fluorescent indicators for Ca^{2+} and Zn^{2+}. It involves a transient interaction between the excited state of the fluorophore and a halide ion, no ground-state complex is formed. Quenching of quinolinium dyes by other halides, such as bromide and iodide, and other anions, such as thiocyanate, is more efficient than chloride quenching. Fortunately, physiological concentrations of non-chloride ions do not significantly affect the fluorescence of SPQ and other methoxyquinolinium-based chloride indicators.

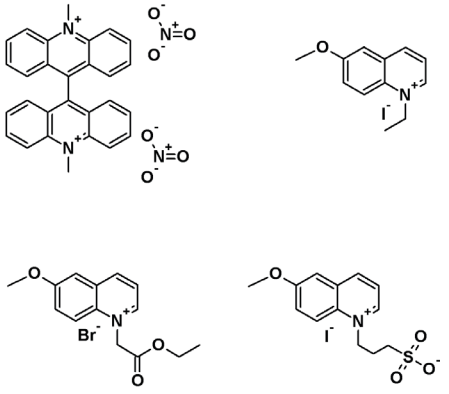

Figure 6.4. The chemical structures of fluorescent chloride indicators: Lucigenin (top left, Cat# 21259), MEQ (top right, Cat# 21250), MQAE (bottom left, Cat# 21255) and SPQ (bottom right, Cat# 21252).

Fluorescence of the quinolinium dyes is quite sensitive to solution viscosity and volume since the chloride-dependent fluorescence quenching is a diffusional process. The efficiency of collisional quenching is characterized by the Stern–Volmer constant (K_{SV}) — the reciprocal of the ion concentration that produces 50% of maximum quenching. In these assays, SPQ- or MQAE-loaded cells are successively perfused with chloride-containing extracellular medium followed by medium in which the chloride content is replaced by nitrate.

SPQ is currently in widespread use for detecting CFTR activity using the chloride/nitrate exchange technique. SPQ has also been employed to investigate chloride fluxes through several other transporters such as the GABA receptor. MQAE has greater sensitivity to chloride and a higher fluorescence quantum yield than SPQ, and consequently MQAE is currently the more widely used of the two indicators. The ester group of MQAE may slowly hydrolyze inside cells, resulting in a change in its fluorescence response. MQAE has been used in a fluorescence microplate assay that has potential for screening compounds that modify chloride channel activity.

Iodide Blue™-Based Chloride Channel Assay

Chloride channels have a variety of important physiological and cellular functions that include regulation of pH, volume homeostasis, organic solute transport, cell migration, cell proliferation and differentiation. Chloride channels represent valuable drug targets. However, the existing technologies for screening chloride channel modulators are a compromise between throughput, sensitivity and physiological relevance. Screen Quest™ Colorimetric Chloride Channel Assay Kit (Cat# 36350) provides an optimized assay method for monitoring chloride channels. The assay uses our proprietary iodide indicator (Iodide Blue™) for measuring iodide concentration, and as low as 30 nM iodide was detected. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

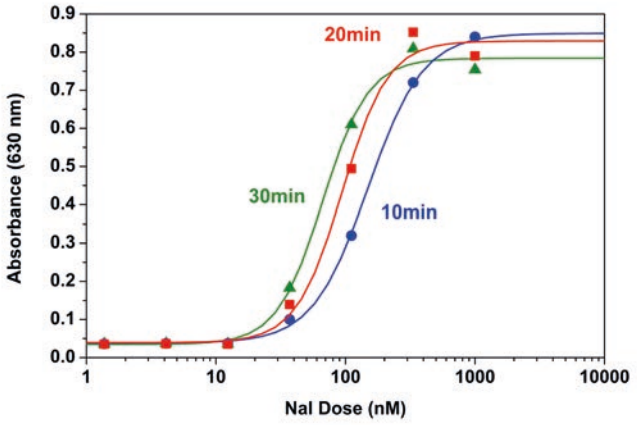

Figure 6.5. NaI dose responses were measured with Screen Quest™ Colorimetric Chloride Channel Assay Kit (Cat# 36350) in a 96-well black wall/clear bottom plate. As low as 30 nM NaI was detected with 10 minutes incubation (n=3).

Table 6.2 Chloride Detection Reagents and Assay Kit

Cat #	Product Name	Size	$K_{sv}(M^{-1})$ (in solution)	$K_{sv}(M^{-1})$ (in cell)	Ex (nm)	Em (nm)
21259	Lucigenin	10 mg	390		455	505
21250	MEQ	100 mg	145	19	344	442
21255	MQAE	100 mg	200	25-28	350	460
36350	Screen Quest™ Colorimetric Chloride Channel Assay Kit	10 plates	N/A	N/A	630	N/A
21252	SPQ	25 mg	118	12	344	433

6.3 Near Neutral pH Measurement

Intracellular pH plays an important modulating role in many cellular events, including cell growth, calcium regulation, enzymatic activity, receptor-mediated signal transduction, ion transport, endocytosis, chemotaxis, cell adhesion, and other cellular processes. pH-sensitive fluorescent dyes have been widely applied to monitor changes in intracellular pH in recent years. Imaging techniques that use fluorescent pH indicators also allow researchers to investigate these processes with much greater spatial resolution and sampling density than what can be achieved using other technologies such as microelectrode. Among them, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) is the most popular pH probe since it can be used to monitor cellular pH ratiometrically. However, all the commercial BCECF AM are a complex mixture of six isomers with different ratios from batch to batch, complicating the BCECF applications.

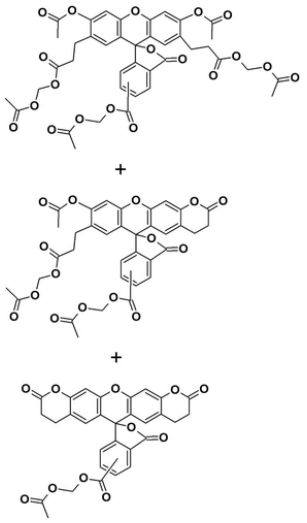

Figure 6.6. Structures of BCECF AM (the complicated mixture of six components).

RatioWorks™ BCFL AM (Cat# 21190) is developed to overcome the isomer difficulty associated with BCECF AM. As BCECF AM, RatioWorks™ BCFL AM exhibits pH-dependent dual excitations, essentially identical to those of BCECF AM. It has a pK_a of ~7.0, identical to BCECF AM, too. As with BCECF AM, the dual excitation spectrum of RatioWorks™ BCFL AM with an isosbestic point at 454 nm should make RatioWorks™ BCFL AM a good excitation-ratiometric pH indicator. RatioWorks™ BCFL ratiometric imaging makes intracellular pH determination essentially independent of several variable factors, including dye concentration, path length, cellular leakage and photobleaching rate. RatioWorks™ BCFL AM is a single isomer, making the pH measurement much more reproducible than BCECF AM, which is consisted of quite a few different isomers.

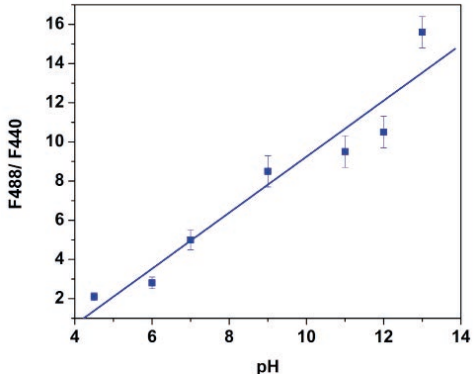

Figure 6.7. The fluorescence excitation ratios of RatioWorks™ BCFL AM (Cat# 21190) at 488 nm and 440 nm were measured with 530 nm emission at pH 4.5, 6, 7, 9, 11, 12 and 13 using standard buffer solutions. The higher the pH, the higher the fluorescence signal with longer excitation wavelength (488 nm), and the lower the fluorescence signal with shorter excitation wavelength (440 nm).

Intracellular pH changes are implicated in diverse physiological and pathological processes, including cell proliferation, apoptosis, fertilization, malignancy, multidrug resistance, ion transport, lysosomal storage disorders and Alzheimer's disease. Cell Meter™ Fluorimetric Intracellular pH Assay Kit (Cat# 21180) utilizes AAT Bioquest's proprietary fluorescent indicator for measuring the relative intracellular pH changes. It is a homogeneous, kinetic, live-cell fluorescent assay that utilizes either a standard procedure or acid-load procedure. The standard protocol is designed for measuring the therapeutic targets of interest with a decrease in intracellular pH upon treatment. The 'Acid-Load' procedure is designed to measure the increase of intracellular pH associated with changes in cellular metabolism due to GPCR activation or growth factor activity. With the 'Acid-Load' procedure ammonium chloride solution is added after the fluorescent pH dye is loaded into cells in a minimum volume. This acid-loading step is followed by the addition of agonist in a relatively large volume (~4X) of buffer. The sudden volume change initiates an efflux of ammonia (NH3) from the cells causing a rapid decrease in intracellular pH, and thus a decrease in fluorescence signal. The effect of agonist on the subsequent recovery of intracellular pH is measured by the relative fluorescence signal increase.

Table 6.3 Fluorescent pH Probes for Near -Neutral pH Environments

Cat #	Product Name	Size	pK_a	Ex (nm)	Em (nm)
21201	BCECF Acid	1 mg	7.0	503	528
21202	BCECF AM	1 mg	7.0	503	528
21203	BCECF AM *UltraPure Grade*	20x50 µg	7.0	505	520
21180	Cell Meter™ Fluorimetric Intracellular pH Assay Kit	500 tests	N/A	503	528
21189	RatioWorks™ BCFL Acid *Superior Replacement to BCECF*	1 mg	7.0	503	528
21190	RatioWorks™ BCFL AM *Superior Replacement to BCECF AM*	1 mg	7.0	503	528
21191	RatioWorks™ BCFL SE *Superior Replacement to BCECF SE*	1 mg	7.0	503	528

6.4 Phosphate (Pi) Detection

Phosphate (Pi) is one of the most important inorganic ions in biological systems. It functions in a variety of roles. One of the most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. Numerous enzymes of therapeutic relevance produce phosphate directly or through coupled reactions. These potential drug development targets include lipid and protein phosphatases, ATPases, GTPases, prenyltransferases and phosphodiesterases. Phosphate is also of great importance in mineralization processes and is a primary stimulus of algal blooms frequently found in bodies of fresh water, due to run-off from areas of high fertilizer use. The importance of phosphate in drug discovery and other fields makes high quality phosphate assays indispensable.

MESG-Based Colorimetric Phosphate Assay

In the presence of inorganic phosphate, MESG is converted to 2-amino-6-mercapto-7-methlpurine by purine nucleoside phosphorylase (PNP) with a red shift of absorption wavelength. This feature has been used to quantify phosphate spectrophotometrically. The enzymatic removal of the ribose moiety from MESG results in a shift in the wavelength of maximum absorbance (λ_{max}) from 330 nm to 360 nm. Because conversion of MESG requires inorganic phosphate, the increase in absorbance at 360 nm can be used to measure phosphate concentration. When the PNP

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enzyme and MESG substrate are in excess relative to phosphate, the increase in absorbance at 360 nm is quantitative for inorganic phosphate. Assuming there is no preexisting phosphate, any increase in the absorbance at 360 nm must be the result of Pi liberation from ATP hydrolysis.

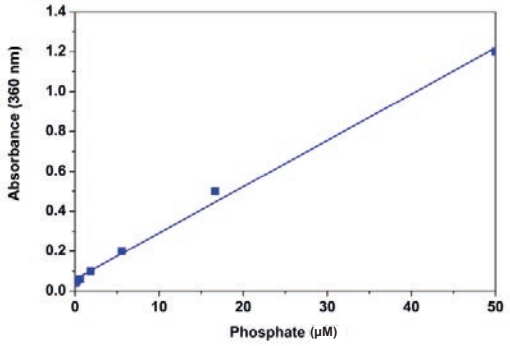

Figure 6.8. Phosphate dose responses were measured with PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit (Cat# 21659) in a 96-well UV plate. As low as 0.2 μM phosphate was detected with 30 minutes incubation.

Malachite Green-Based Colorimetric Phosphate Assay

PhosphoWorks™ Colorimetric Phosphate Assay Kit (Cat# 21665) has been developed for measuring the activity of any Pi-generating enzyme through the complexation of malachite green with phosphate under acidic conditions. The measurement of Pi is based on the change in absorbance of the malachite green derivative in the presence of molybdate. This assay kit is formulated to give sensitive detection of Pi, providing an alternative to hazardous radioactive methods and other less sensitive colorimetric assays. Unlike other malachite dye formulations, PhosphoWorks™ Colorimetric Phosphate Assay Kit gives a completely stable end-point signal.

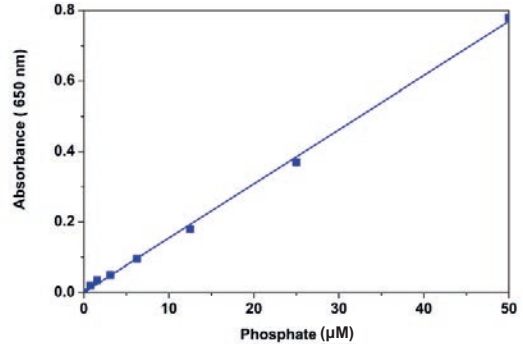

Figure 6.9. Phosphate dose responses were measured with the PhosphoWorks™ Colorimetric Phosphate Assay Kit (Cat# 21665) in a 96-well clear bottom plate. As low as 0.1 μM of phosphate was detected with 10 minutes incubation.

Fluorimetric Phosphate Assay

Detection of many phosphoester–metabolizing enzymes is difficult because suitable substrates are not available. It usually has been necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods. We have developed the PhosphoWorks™ Fluorimetric Phosphate Assay Kit (Cat# 21660) for measuring the activity of any Pi-generating enzyme using our red fluorescent phosphate sensor. The measurement of Pi is based on the change in the absorbance and fluorescence of the phosphate sensor. The assay is shown to quantify phosphate in solution at the concentration as low as 0.1 μM. It can be used to measure the kinetics of phosphate release from phosphatases (such as GTPases and ATPases) by coupling the two enzymatic reactions. The kit provides sensitive detection of Pi, an alternative to hazardous radioactive methods and other less sensitive colorimetric assays. It comes with all the essential reagents including phosphate sensor, phosphate standards and assay buffer. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

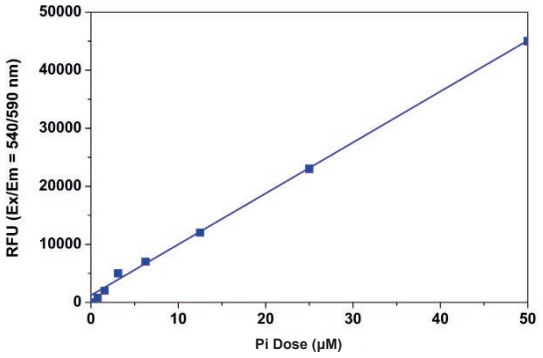

Figure 6.10. Phosphate dose responses were measured with PhosphoWorks™ Fluorimetric Phosphate Assay Kit (Cat# 21660) in a 96-well black plate. As low as 0.1 μM phosphate was detected with 1 hour incubation.

Table 6.4 Phosphate Detection Reagent and Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
21600	MESG *Phosphate assay reagent*	5 mg	330	N/A
21659	PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit *UV absorption*	200 tests	360	N/A
21665	PhosphoWorks™ Colorimetric Phosphate Assay Kit *Blue Color*	1,000 tests	650	N/A
21660	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Red Fluorescence*	100 tests	571	585

6.5 Pyrophosphate (PPi) Assay

Pyrophosphate (PPi) is produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation by the enzyme adenylate cyclase and the enzymatic activation of fatty acids to form the coenzyme A esters. Phospho-

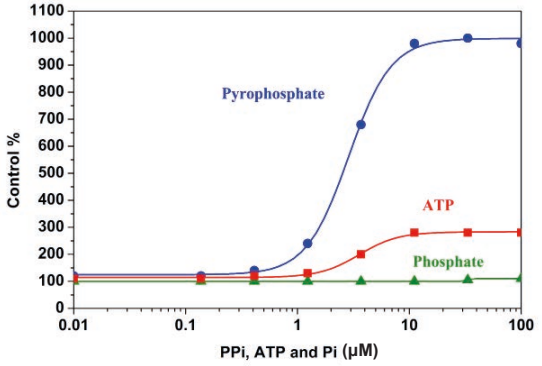

Figure 6.11. Pyrophosphate and phosphate dose responses were measured with the PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit (Cat# 21611) in a 96-well black solid plate. As low as 1 μM (100 picmoles/well) pyrophosphate was detected with 10 minutes incubation.

Works™ Fluorimetric Pyrophosphate Assay Kits (Cat# 21611 and 21614) provide the most robust spectrophotometric method for measuring pyrophosphate. They use our proprietary fluorogenic pyrophosphate sensors that have the fluorescence intensity proportionally dependent upon the concentration of pyrophosphate. The assay is much easier than the enzyme-coupling pyrophosphate methods that require at least two enzymes for the pyrophosphate detections. PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kits provide all the essential components for assaying pyrophosphate. They have been successfully used in high throughput screening (HTS).

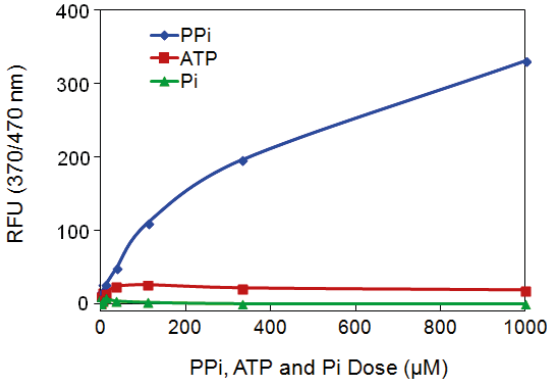

Figure 6.12. Pyrophosphate, ATP and phosphate dose responses were measured with PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit (Cat# 21614) in a 96-well black solid plate using a fluorescence microplate reader. As low as 1 μM (100 picmoles/well) pyrophosphate was detected with 10 minutes incubation.

Table 6.5 Pyrophosphate Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
21611	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Blue Fluorescence*	200 tests	316	456
21614	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Enhanced Selectivity*	200 tests	370	467

6.6 Zinc Ion Assays

Zinc is an essential trace mineral element that plays an important role in a number of biological processes. It is an essential factor required for many enzymes, protein structures, and control of genetic expression. Zinc status also affects basic processes of cell division, growth, differentiation, development, and aging. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhea, poor healing, stunting, hypogonadism, fetal growth failure, and teratology. Simple, direct and automation-ready procedures for measuring zinc ion are highly desirable in research and drug discovery.

Amplite™ Colorimetric Zinc Ion Quantitation Kit (Cat# 19001) provides a robust method for detecting zinc concentration in biological samples using our proprietary Zn-620™, in which zinc binds to the probe with the enhanced absorption at around 620 nm. In zinc-free solution, the absorbance is at 480 nm. However, when zinc ions bind to the probe, the absorbance exhibits a large increase at 620 nm (>100 folds). The concentration of zinc can be measured colorimetrically at the absorbance ratio of A_{610nm}/A_{480nm}. The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 2 μM (130 ng/mL) zinc ion.

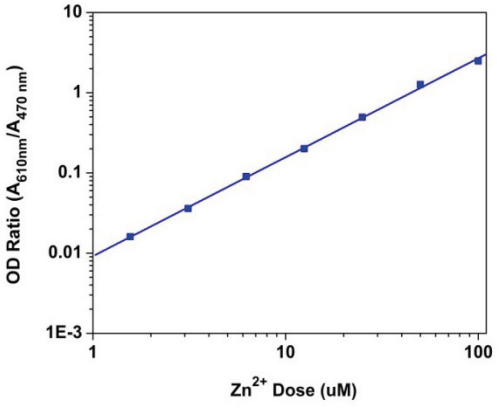

Figure 6.13. Zn^{2+}dose response was measured on a 96-well clear bottom plate with Amplite™ Colorimetric Zinc Ion Quantitation Kit (Cat# 19001). As low as ~ 2 μM of Zn^{2+} was detected with 5 minutes incubation.

Amplite™ Fluorimetric Zinc Ion Quantitation Kit (Cat# 19000) provides a robust method for detecting zinc concentration in biological samples using our proprietary Metal Fluor™ Zn 520, in which zinc binds to the probe with enhanced fluorescence at Ex/Em= 485/525 nm. The zinc probe exhibits a large increase in fluorescence in response to Zn^{2+} (greater than 200~300 folds). It has high Zn^{2+}-specificity with little responses to other metals, including Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}. The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 0.2 μM (13 ng/mL) zinc ion.

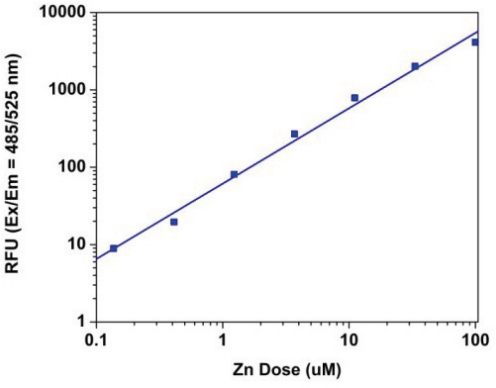

Figure 6.14. Zn^{2+} dose responses were measured on a 96-well black plate with Amplite™ Fluorimetric Zinc Ion Quantitation Kit (Cat# 19000). As low as ~ 0.2 μM Zn^{2+} was detected with 5 minutes incubation (n=3).

Table 6.6 Zinc Ion Quantitation Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
19001	Amplite™ Colorimetric Zinc Ion Quantitation Kit	200 tests	620	N/A
19000	Amplite™ Fluorimetric Zinc Ion Quantitation Kit	200 tests	492	514

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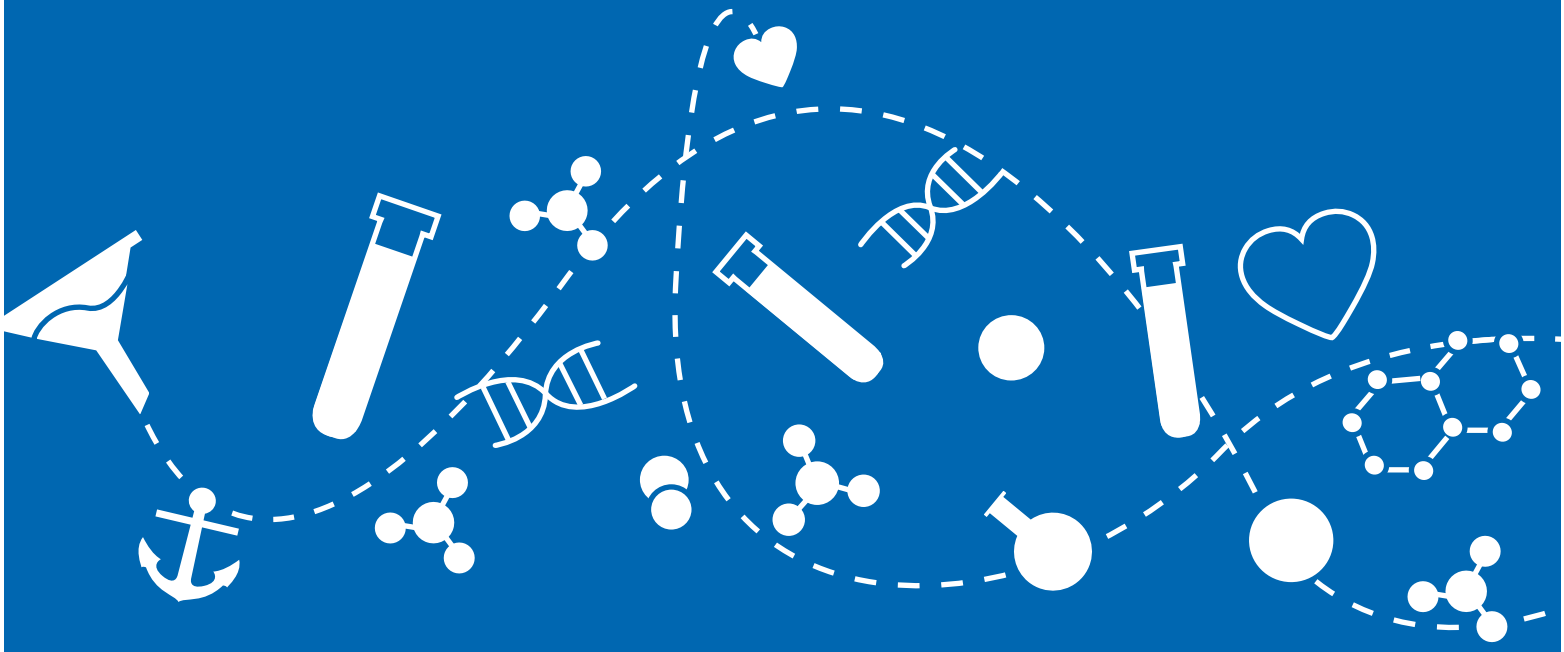

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