Cell Metabolism and Signaling Assays

Cell Metabolites | Cell Signaling Molecules

AAT Bioquest®

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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TERMS AND CONDITIONS OF SALE

1. Prices, Orders and Changes: Prices shown are in US currency. Please call us for current prices if you require this information prior to placing your order. We guarantee our written quotations for 60 days. You may not cancel purchase orders unless such cancellation is expressly agreed by us. In such event, you will be advised of the total charge for such cancellation. You agree to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on us by our suppliers, and any other cost resulting from cancellation of this order.

2. Delivery: In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

4. Warranties: The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

5. Returns: We must authorize any returns. We will not accept return shipments unless we have given prior written permission and shipping instructions. Goods may not be returned for credit except with our permission, and then only in strict compliance with our return shipment instructions. Any returned items may be subject to a 20% restocking fee. In many cases, items ordered in error cannot be returned because of the sensitive nature of many of our products and the difficulty and expense of requalifying returned items. If items are accepted for return, they must be in new, unopened, unused and undamaged condition, and you will be charged a per-unit 20% restocking charge.

6. Use of Our Products: Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

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8. Miscellaneous: We reserve the right to discontinue our products or change specifications or prices of our products and to correct any errors or omissions at any time without incurring obligations.

Quantifying Biomolecules

A biomolecule is a term loosely applied to molecules and ions present in all organisms. These include organic macromolecules such as proteins, carbohydrates, lipids, and nucleic acids, as well as small molecules such as macromolecular building blocks, cell metabolites, cell signaling molecules, pharmaceutical compounds, and ions. While biomolecules may vary widely in size and structure, they are essential to many biological processes including cell division, cell metabolism, homeostasis, growth and development.

Accurate determination of the presence and amount of biomolecules is important for any research application. For example, in colorimetric assays it is important to start with the appropriate amount or concentration of a biomolecule. If the initial sample concentration being used is too high, the assay will become saturated and results will be inaccurate. A solution then would be to dilute the sample. In clinical settings, biomolecular quantification can be used to verify panels of biomarkers with potential pathological impact. Furthermore, in drug discovery, quantification is often used for profiling metabolites of pharmaceutical compounds, establishing rate of drug degradation, or identifying undesirable side effects.

Quantitative analysis of biomolecules can be performed using various detection formats including colorimetric (absorbance), fluorescence and chemiluminescence platforms. Each detection solution has its own advantages (**Table 1**). Colorimetric quantification assays are cost effective, easy to use, noninvasive and capable of revealing the presence of potential sample contaminants. Fluorescence quantification assays are highly sensitive and specific with the capacity to accurately measure biomolecules over a wide concentration range. Chemiluminescence quantitative analysis minimizes autofluorescence in biological samples by eliminating the use of an excitation source. Chemiluminescence signals are generated via chemical reaction. The following section discusses our numerous reagents and assays for conducting quantitative analysis of biomolecules in a wide variety of biological samples including cell lysates and cell-based assays.

Table 1. Summarization of colorimetric, fluorescence and chemiluminescence detection formats.

Format	Reagents	Instruments	Principle	Advantages
Colorimetric Detection	• Enzyme conjugate (e.g. HRP-labeled antibody) • Chromogenic substrate (e.g. 4CN or DAB)	• Absorbance microplate readers	Chromogenic substrate is catalyzed by an enzyme conjugate to produce an easily visible colored precipitate. Measuring the amount of light absorbed by chromogenic reaction product can be quantitatively correlated to the analyte concentration.	• Colorimetric assay components are economical. • Noninvasive, requires no direct manipulation of the material being measured. • Provides indication of sample contaminants.
Fluorescence Detection	• Fluorophore-labeled conjugate (e.g. iFluor™488-goat anti-mouse IgG)	• Fluorescence microplate readers • Fluorescence microscopes • Flow cytometers	A fluorophore labeled conjugate is used to detect analyte. A light source excites the fluorophore, which then emits a fluorescent signal. The signal intensity generated is directly proportional to analyte concentration.	• High sensitivity due to high extinction coefficients of fluorophores. • High target specificity makes technique ideal for samples containing contaminants. • Wide dynamic range and greater limits of detection. • Adaptable for multiplexing applications.
Chemiluminescence Detection	• Enzyme conjugate (e.g. Luciferase-labeled antibody) • Chromogenic substrate (e.g. luciferin)	• CCD Camera • X-Ray film • Luminescence microplate readers	Chemical substrate is catalyzed by an enzyme to produce light as a by-product. The light intensity measured can be quantitatively correlated to the analyte concentration.	• Relatively simple instrumentation required. • Very low detection limits and wide dynamic ranges. • Minimal background interference.

Amino Acids, Peptides and Proteins

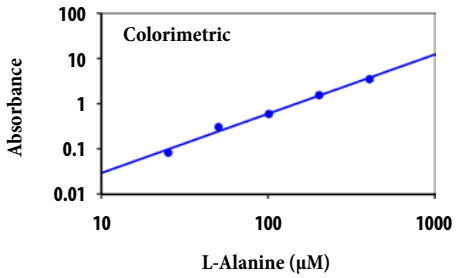

Figure 2.1 L-alanine dose response was measured with Amplite™ Colorimetric L-Alanine Assay Kit (Cat#13826) on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 10 µM L-alanine can be detected with 30 minutes incubation at 37 °C

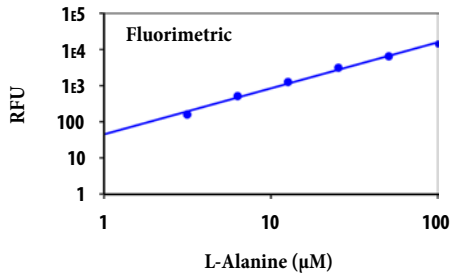

Figure 2.2 L-alanine dose response was measured with the Amplite™ Fluorimetric L-Alanine Assay Kit (Cat# 13825) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 1.5 µM L-alanine can be detected with 30 minutes incubation at 37 °C.

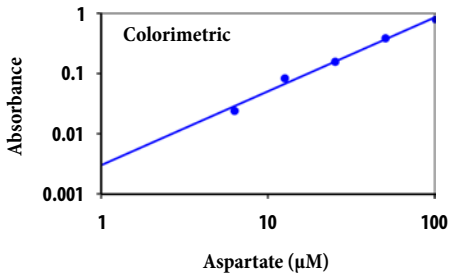

Figure 2.3 Aspartate dose response was measured with Amplite™ Colorimetric Aspartate Assay Kit (Cat#13828) on a clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 6.25 µM aspartate was detected with 20-30 minutes incubation.

Amino acids, twenty in all, are the building blocks for large macromolecules known as proteins. They link together via peptide bonds to form chains of amino acid residues varying in length and sequence. As these chains form, the polypeptide develops multiple levels of structure which contribute to its overall shape and functionality. Common examples of proteins include enzymes, antibodies and structural and transport proteins.

Quantitative amino acid analysis is a useful tool for many scientific applications. Since proteins and amino acids are essential for cellular repair and synthesis, amino acid quantification can be used to determine the metabolic states of cells by analyzing free amino acid concentrations of biological samples. Additionally, characterization of recombinant proteins, concentration content, molar ratio and extinction coefficients may be determined using amino acid analysis. This section highlights our reagents and assays for conducting quantitative amino acid analysis using colorimetric or fluorimetric detection platforms.

L-Alanine Assays

L-alanine (L-Ala) is a α-amino acid and a key building block in the biosynthesis of proteins. It is synthesized primarily by myocytes from lactic acid and absorbed via the liver. L-alanine can also be synthesized from pyruvate and branched amino acids such as valine and leucine. In the liver L-alanine is converted into pyruvate by glutamic-pyruvic transaminase before entering the metabolic mainstream. L-alanine is essential for the synthesis of glucose and thus blood sugar management, and plays a vital role in immune system response and the prevention of kidney stones. Measuring L-alanine levels is a useful biomarker for assessing poor nutrition, low protein diet and stress.

Our Amplite™ Colorimetric L-Alanine assay provides a convenient and robust colorimetric assay for the quantification of L-alanine in biological samples. It utilizes an enzyme-coupled reaction that releases H_2O_2, which can be measured with our Quest Fluor™ L-Alanine Sensor. In this assay, as little as 10 µM of L-alanine can be detected using an absorbance microplate reader at 575 nm. Our Amplite™ Fluorimetric L-Alanine assay utilizes a similar approach for measuring L-alanine. This assay has the capacity to detect as little as 1.5 µM of L-alanine using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Aspartate Assays

Aspartate, or aspartic acid, is a negatively charged, polar α-amino acid. It is synthesized primarily from oxaloacetate via transamination and is involved in many biochemical roles. It is a metabolite in the urea cycle and participates in the metabolic pathway gluconeogenesis. It is a precursor to several other amino acids and hormones, and acts as a neurotransmitter stimulating NMDA receptors.

Amplite™ Colorimetric Aspartate assay provides a simple and sensitive assay for

the quantification of aspartate in biological samples. In this assay, aspartate is first converted to pyruvate, and then an enzyme coupled reaction is utilized to generate H_2O_2. The level of H_2O_2 produced is detected with our Amplite™ Red substrate. In this assay, as little as 6.25 µM of aspartate can be detected using an absorbance microplate reader at 575 ± 5 nm. Our Amplite™ Fluorimetric Aspartate assay utilizes a similar approach for determining aspartate. It has the capacity to detect as little as 0.4 µM of aspartate using a fluorescence microplate reader at Ex/Em = 540/590 nm.

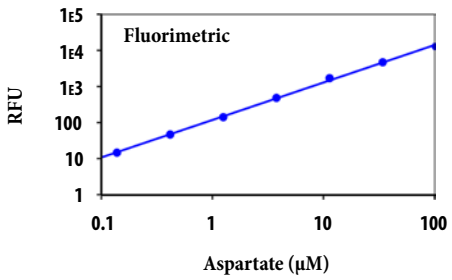

Figure 2.4 Aspartate dose response was measured with Amplite™ Fluorimetric Aspartate Assay Kit (Cat#13827) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.4 µM aspartate can be detected with 20-30 minutes incubation.

Glutamic Acid Assays

Glutamic acid is a nonessential α-amino acid used in the biosynthesis of proteins. The salts and carboxylate anions of glutamic acid are referred to as glutamates. Glutamates are key compounds utilized in many cellular mechanisms. Glutamic acid is the most abundant excitatory neurotransmitter in the mammalian nervous system and it plays an important role in synaptic plasticity and cognitive functions. It is a precursor for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and is a key compound in cellular metabolism. Since glutamic acid is one of few nutrients capable of passing the blood-brain barrier, it acts as a promising therapeutic target in the treatment of diseases such as depression, ADD, muscular dystrophy, mental retardation and psychiatric disorders.

Amplite™ Fluorimetric Glutamic Acid assay offers a rapid and robust method for the quantification of glutamic acid in biological samples. This assay employs an enzyme-coupled system that catalyzes the reaction between L-glutamic acid and NADP to generate NADPH. The level of NADPH produced is recognized by a proprietary NADPH sensor and then recycled back to NADP. In this assay, as little as 10 µM of glutamic acid can be detected using either a fluorescence microplate reader at Ex/Em = 540/590 nm, or an absorbance microplate reader at the absorbance ratio of $A_{570\ nm}/A_{605\ nm}$.

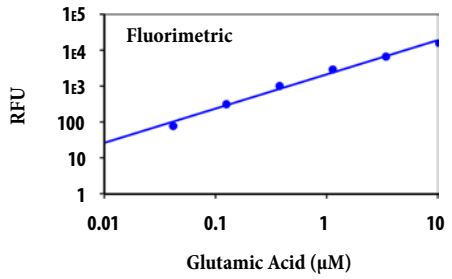

Figure 2.5 Glutamic acid dose response was measured with Amplite™ Fluorimetric Glutamic Acid Assay Kit (Cat#10054) in a black 96-well plate using a Gemini (Molecular Devices) microplate reader. As low as 10 µM glutamic acid was detected with 1 hour incubation.

Glutathione Assays

Glutathione (GSH) is a tripeptide comprised of the amino acids L-cysteine, L-glutamic acid and glycine. It is involved in the development and maintenance of disulfide bonds in proteins, the transport of amino acids across cell membranes, and in detoxification. Since glutathione contains the crucial thiol group, it serves as one of the major endogenous antioxidants in cells preventing damage by neutralizing reactive oxygen species such as free radicals and peroxides. Monitoring reduced and oxidized glutathione in biological samples is a useful tool for evaluating the redox and detoxification status of cells and tissues against oxidative and free radical mediated cell injury. While few reagents and assay kits are commercially available for quantifying glutathione, they are hindered by their lack of sensitivity and tedious protocols.

Amplite™ Fluorimetric Glutathione assay offers a convenient and ultra-sensitive method for the quantification of glutathione in biological samples. This assay employs a non-fluorescent, pH-dependent glutathione sensor called Thiolite™ Green, which

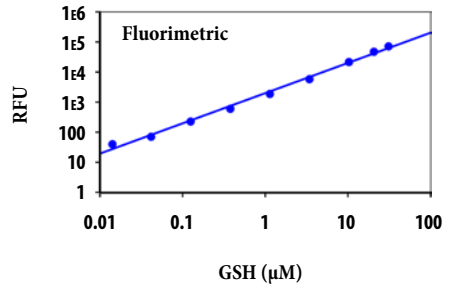

Figure 2.6 GSH dose responses was measured in a black 96-well plate with Amplite™ Fluorimetric Glutathione Assay Kit (Cat#10055) using a NOVOstar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH was detected with 10 minutes incubation (n=3).

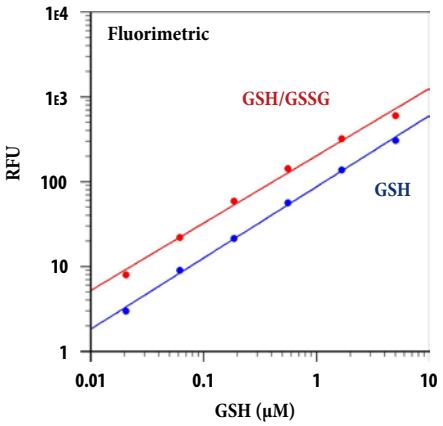

Figure 2.7 GSH and Total GSH (GSH + GSSG) dose responses were measured with Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat# 10056). Blue line: in the presence of GSH only. Red line: in the presence of 1:1 GSH/GSSG.

upon association with glutathione generates a strong green fluorescence. In this assay, as little as 10 nM of glutathione can be detected using a fluorescence microplate reader at Ex/Em = 490/520 nm. It is convenient in 96-well or 384-well microtiter-plate format and is easily adaptable for automation without a separation step.

Glutathione GSH/GSSG Ratio Assays

In healthy cells and tissues, glutathione exists in two states, reduced (GSH) and oxidized (GSSG). In the reduced state, GSH is a major tissue antioxidant that neutralizes reactive oxygen species. As a result of donating an electron, GSH itself becomes reactive and will readily react with other reactive GSH molecules to form GSSG. Additionally, reduced GSH 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 forms GSSG. The enzyme glutathione reductase (GR) recycles GSSG to GSH while simultaneously oxidizing β-nicotinamide adenine dinucleotide phosphate (β-NADPH2). When cells are exposed to increased levels of oxidative stress, GSSG accumulates and the ratio of GSH/GSSG increases. As such, monitoring GSH/GSSG levels in biological samples has become a useful tool in evaluating oxidative stress of cells and tissues, and cell injuries caused by free radicals.

Amplite™ Fluorimetric Glutathione GSH/GSSG assay offers a convenient and ultra-sensitive method for the quantification of GSH in biological samples such as plasma, urine and cell lysates. This assay employs a non-fluorescent proprietary sensor, which upon reacting with GSH fluoresces strongly. In this one-step fluorimetric assay, as little as 10 nM of GSH or GSSG can be detected using a fluorescence microplate reader at Ex/Em = 490/520 nm. It is convenient in 96-well or 384-well microtiter-plate format and is easily adaptable for automation without a separation step.

Product Ordering Information For Quantifying Amino Acids, Peptides and Proteins

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
13826	Amplite™ Colorimetric L-Alanine Assay Kit	200 Tests	575	N/A
13828	Amplite™ Colorimetric L-Aspartate (Aspartic Acid) Assay Kit	200 Tests	575	N/A
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
13825	Amplite™ Fluorimetric L-Alanine Assay Kit	200 Tests	571	585
13827	Amplite™ Fluorimetric L-Aspartate (Aspartic Acid) Assay Kit	200 Tests	571	585

Lipid Biology

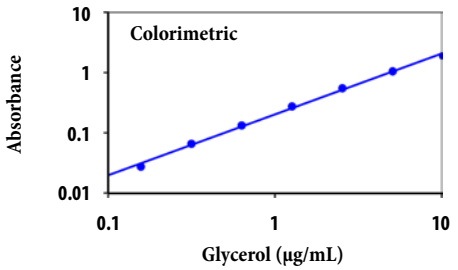

Figure 3.1 Glycerol dose response was measured with Amplite™ Colorimetric Glycerol Assay Kit (Cat#13832) on a black wall/clear bottom 96-well plate using a SpectraMax reader. As low as 0.15 µg/mL (~1.6 µM) glycerol was detected with 30 minutes incubation.

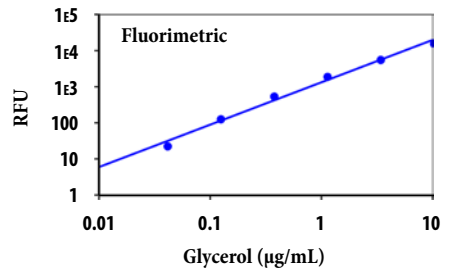

Figure 3.2 Glycerol was measured with Amplite™ Fluorimetric Glycerol Assay Kit (Cat#13833) on a solid black 96-well plate using a Gemini microplate reader. As low as 0.015 µg/mL (~0.16 µM) glycerol was detected with 20 minutes incubation.

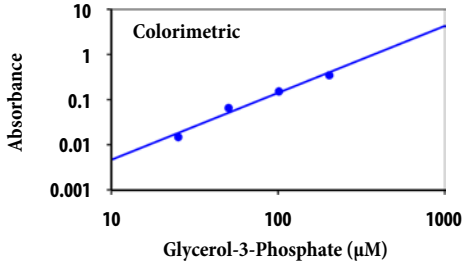

Figure 3.3 G3P dose response was obtained with Amplite™ Colorimetric G3P Assay Kit (Cat#13838) in a 96-well clear bottom /black wall plate using a Spectra Max absorbance microplate reader (Molecular Devices). As low as 12.5 µM of G3P can be detected with 30 minutes incubation time (n=3).

Lipids are a class of hydrophobic biomolecules that play key roles in biological functions such as the structural components of cell membranes, energy storage, cell signaling and as precursors for certain hormones. The primary components that make up lipid molecules are fatty acids, sterols and glycerol. Common examples of lipids are phospholipids, triglycerides, steroids and waxes.

Quantitative analysis of lipid building blocks is a powerful tool in lipidomics, the study of pathways and networks of cellular lipids in biological systems. Since lipid metabolism is linked to many severe diseases such as diabetes, atherosclerosis and cancer-related diseases, defining the underlying biochemical mechanisms of lipid-related diseases through determination of alterations in cellular lipid signaling or metabolism, can facilitate diagnosis of disease states and treatment efficacy.

Glycerol Assays

Glycerol is a simple polyol compound that is used primarily as a precursor for the synthesis of triglycerides and phospholipids in liver and adipose tissue. During fasting, triglycerides stored within lipid droplets are hydrolyzed to produce glycerol and fatty acids which are released into the blood stream. The amount of free glycerol released into the bloodstream is proportional to the triglyceride/fatty acid cycling rate, which is important in metabolic regulation and heat production.

Amplite™ Colorimetric Glycerol assay provides a simple and robust method for the quantification of glycerol in biological samples. This assay utilizes an enzyme-coupled reaction that oxidizes glycerol to produce H_2O_2. The level of H_2O_2 produced is detected with our Amplite™ Red HRP substrate in the HRP-coupled reaction. In this assay, as little as 1.6 µM of glycerol can be detected using an absorbance microplate reader at the absorbance ratio of $A_{570\ nm}/A_{610\ nm}$. Our Amplite™ Fluorimetric Glycerol assay utilizes a similar approach for measuring glycerol. It has the capacity to detect as little as 0.16 µM of glycerol using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Glycerol 3-Phosphate Assays

Glycerol 3-phosphate (G3P) is a phosphoric ester of glycerol that can be synthesized by reducing the glycolysis intermediate dihydroxyacetone phosphate (DHAP) with glycerol 3-phosphate dehydrogenase. It can also be produced in the liver via glycerol phosphorylation by glycerol kinases. In animals, fungi and plants, G3P is used in the production of ATP. Animals also use G3P in the regeneration of the coenzyme nicotinamide adenine dinucleotide (NAD^+), by neurons and myocytes through the G3P shuttle. Monitoring G3P levels is a useful marker for assessing lipid metabolism and lipid imbalance diseases, such as obesity.

Amplite™ Colorimetric Glycerol 3-Phosphaste assay is an ultra-sensitive method for the quantification of glycerol 3-phosphate in biological samples. This assay utilizes a cycle of enzyme coupling reactions that oxidizes G3P and produces H_2O_2. The level of H_2O_2 generated, which is proportional to the concentration of G3P, is measured with our Amplite™ Red substrate. In this assay, as little as 12.5 µM of G3P can be detected

using an absorbance microplate reader at 576 ± 5 nm. It is convenient for 96-well or 384-well microtiter plate format and its optimized "mix and read" format is suitable for HTS applications. Our Amplite™ Fluorimetric Glycerol 3-Phosphate assay utilizes a similar approach with the capacity to detect as little as 0.14 µM of glycerol using a fluorescence microplate reader at Ex/Em = 540/590 nm.

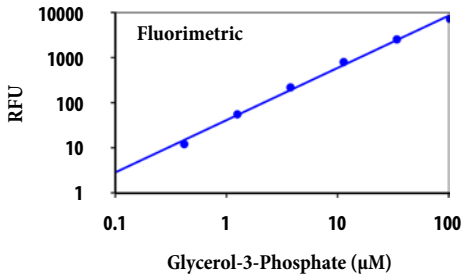

Figure 3.4 G3P dose response was obtained with Amplite™ Fluorimetric Glycerol 3-Phosphate Assay Kit (Cat#13837) in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.41 µM glycerol 3-phosphate can be detected with 30 minutes incubation time (n=3).

Cholesterol Assays

Cholesterol is a sterol molecule biosynthesized by all animal cells. It is an essential structural component of cell membranes and in the maintenance of membrane integrity and fluidity. Cholesterol also serves as a precursor of steroid hormones such as glucocorticoids and gonadocorticoids, bile acid and vitamin D.

Amplite™ Cholesterol Quantitation assay offers a sensitive and robust method for the quantification of cholesterol in biological samples. This assay utilizes an enzyme-coupled reaction for the detection of free cholesterol. Cholesterol is oxidized by cholesterol oxidase to yield H_2O_2. The level of H_2O_2 generated, which is proportional to the concentration of cholesterol, is then detected with our Amplite™ Red substrate. In the presence of peroxidases, Amplite™ Red reacts with H_2O_2 to generate a strong fluorescence. In this assay, as little as 0.03 µM of cholesterol can be detected using a fluorescence microplate reader at Ex/Em = 540/590 nm.

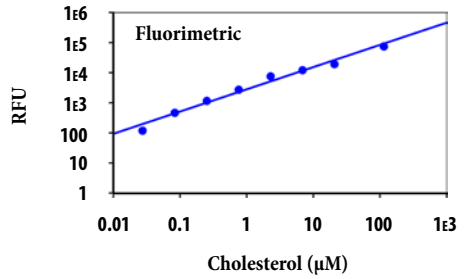

Figure 3.5 Cholesterol dose response was measured with Amplite™ Cholesterol Quantitation Kit (Cat#40006) in a black 96-well plate using a Gemini fluorescence microplate reader (molecular devices). As low as 0.03 µM cholesterol can be detected with 30 minutes incubation (n=3).

Sphingomyelin Assays

Sphingomyelin (SM) is a type of sphingolipid found predominantly in the exoplasmic leaflet of cell membranes. In neurons, it is most abundant in the membranous myelin sheath that surrounds the axons where it plays a vital role in signal transduction. Sphingomyelin also has an important functional role as a precursor of ceramide, a key mediator in signaling pathways. In clinical biology, monitoring sphingomyelin levels is a useful biomarker for assessing Niemann-Pick disease, a lipid storage disorder resulting in the harmful accumulation of sphingomyelin in the spleen, liver and brain.

Amplite™ Fluorimetric Sphingomyelin assay offers a convenient and sensitive method for the quantification of neutral SM and for screening SM inhibitors in biological samples such as blood, cell lysates and solution. In this enzyme-coupled assay, sphingomyelin is hydrolyzed by sphingomyelinase to yield phosphorylcholine. The level of phosphorylcholine produced, which is proportional to the concentration of sphingomyelin, is measured using our Amplite™ Red fluorogenic probe. This assay can detect as low as 1 µM of sphingomyelin using a fluorescence microplate reader at Ex/Em = 540/590. Since this assay is continuous and requires no separation step, it can be readily adapted for automation.

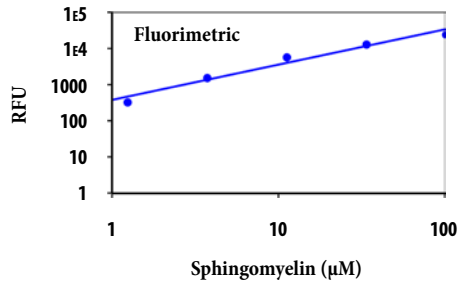

Figure 3.6 Sphingomyelin dose response was measured on a solid black 96-well plate with Amplite™ Fluorimetric Sphingomyelin Assay Kit (Cat#13625) using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 µM sphingomyelin can be detected with 60 minutes incubation (n=3). Note: The fluorescence background increases with time. It is important to subtract the fluorescence intensity value of the blank wells for each data point.

Lipid Droplet Assays

Lipid droplets, or adiposomes, are lipid-rich cellular organelles responsible for regulating the storage and hydrolysis of neutral lipids. These organelles are present predominately in adipose tissue where they serve as lipid reservoirs supplying fatty

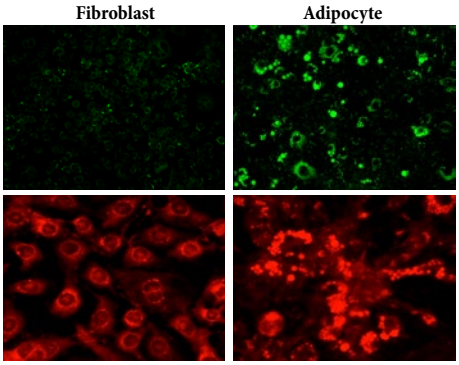

Figure 3.7 Fluorescence images of intracellular lipid droplets in 3T3-L1 Fibroblast (Left) and Adipocyte cells (Right) using Cell Navigator™ Lipid Droplets Fluorescence Assay Kit in green (Cat#22730) and red (Cat#22735) fluorescence. The green fluorescence signal was measured using a fluorescence microscope with a FITC filter. The red fluorescence signal was measured using a fluorescence microscope with a TRITC filter.

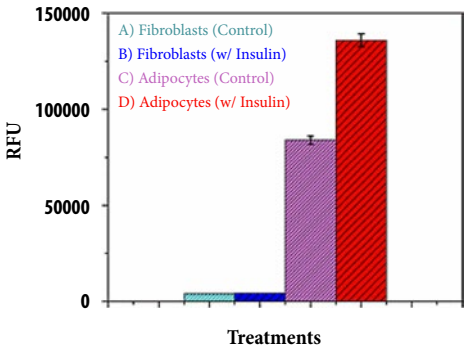

Figure 3.8 Comparison of fatty acid uptake by 3T3-L1 adipocytes and fibroblast. Cells were plated at 50,000 cells/100 µL/well in a 96 well black wall/clear bottom poly-D lysine plate for 5 hours, and then serum deprived for 1 hour. Cells were treated without (control) or with insulin (150 nM), and incubated at 37 °C, 5% CO_2 incubator for 30 min. At the end of the incubation time, 100 µl of fatty acid mixture was added into the well, and incubated for another 60 min, the fluorescence signal was measured with a FlexStation plate reader using bottom read mode. A – fibroblasts (Control); B – fibroblasts (Insulin); C –adipocytes (Control); D– adipocytes (Insulin)

acids and cholesterols for cellular processes, energy, and membrane formation and maintenance. Since an abnormal accumulation of cytoplasmic lipid droplets has been shown to be associated with metabolic diseases such as fatty liver and atherosclerosis, it serves as a useful biomarker for assessing their progression.

Cell Navigator™ Fluorimetric Lipid Droplet assays offer a convenient method for the quantification of lipid droplets in biological samples. These assays utilize either of our lipophilic stains, Nile Green™ or Droplite™ Red to evaluate lipid droplet accumulation. In aqueous media, both Nile Green™ and Droplite™ Red exhibit minimal fluorescence. Upon association with intracellular lipid droplets, both lipophilic stains generate intense fluorescence that can be detected using a fluorescence microscope, flow cytometer or fluorescence microplate reader. Nile Green™ generates a green fluorescence signal that can be read at Ex/Em = 485/520 nm and observed using a FITC filter set. Droplite™ Red generates a red fluorescence signal that can be read at Ex/Em = 550/640 nm and observed using a TRITC filter set.

Fatty Acid Uptake Assays

Fatty acid uptake is a promising therapeutic target for developing novel treatments of many human metabolic-disorders, such as obesity, type-2 diabetes, and hepatic stenosis. For example, in drug discovery, quantitative analysis of fatty acid uptake in cells containing fatty acid transporters makes possible the characterization of agonist and antagonists effects of novel drug compounds.

Screen Quest™ Fluorimetric Fatty Acid Uptake assay provides a simple method for monitoring fatty acid uptake in cells known to contain fatty acid transporters. This assay utilizes our proprietary dodecanoic acid fluorescent fatty acid substrate. It can be performed on any fluorescence microplate reader with a bottom-read mode at Ex/Em = 485/515 nm or FITC channel. The assay can be performed in 96-well or 384-well microtiter plate in a simple mix and-read procedure, and easily adapted for high throughput screening applications.

Product Ordering Information For Lipid Biology Quantification

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
40006	Amplite™ Cholesterol Quantitation Kit	200 Tests	571	585
13838	Amplite™ Colorimetric Glycerol 3-Phosphate (G3P) Assay Kit	200 Tests	575	N/A
13832	Amplite™ Colorimetric Glycerol Assay Kit	200 Tests	575	N/A
13837	Amplite™ Fluorimetric Glycerol 3-Phosphate (G3P) Assay Kit	200 Tests	571	585
13833	Amplite™ Fluorimetric Glycerol Assay Kit	200 Tests	571	585
13625	Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*	100 Tests	571	585
22730	Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit *Green Fluorescence*	500 Tests	485	520
22735	Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit *Red Fluorescence*	500 Tests	550	640
36385	Screen Quest™ Fluorimetric Fatty Acid Uptake Assay Kit	100 Tests	490	515

Carbohydrate Metabolism

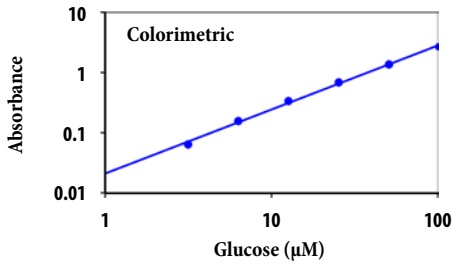

Figure 4.1 Glucose oxidase dose response was measured with Amplite™ Colorimetric Glucose Oxidase Assay Kit (Cat#11299) on a 96-well clear bottom plate using a SpectraMax reader (Molecular Devices).

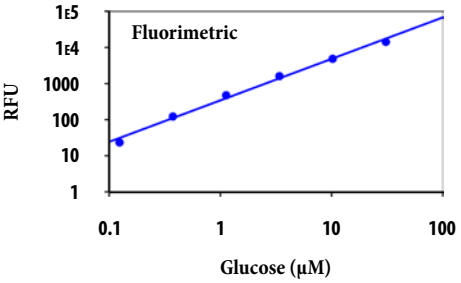

Figure 4.2 Glucose dose response was measured with Amplite™ Fluorimetric Glucose Quantitation Kit (Cat #40005) on a 96-well black plate using a Gemini microplate reader (Molecular Devices). As low as 0.1 µM glucose was detected with 30 minutes incubation (n=3).

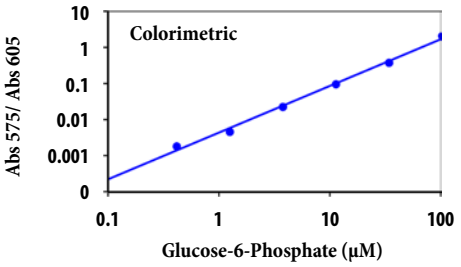

Figure 4.3 G6P dose response was measured with Amplite™ Colorimetric G6P Assay Kit (Cat#13805) in a 96-well white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 1 µM G6P in 100 µL volume can be detected with 1 hour incubation.

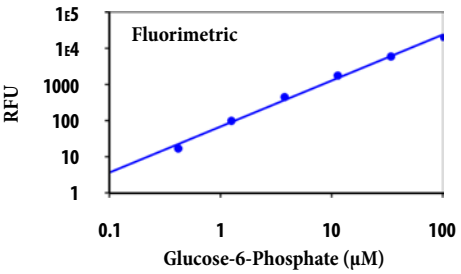

Figure 4.4 G6P dose response was measured with Amplite™ Fluorimetric G6P Assay Kit (Cat#13804) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 0.3 µM G6P in 100 µL volume can be detected with 1 hour incubation.

Carbohydrates, or saccharides, are another large class of organic biomolecules present in living organisms. They perform numerous roles including a source and storage of energy, a primary structural component in plants, a backbone in genetic molecules and a component of coenzymes. They are categorized as either being monosaccharides, disaccharides or polysaccharides. Monosaccharides and disaccharides are the simplest of carbohydrates and are often referred to as sugars. Polysaccharides are polymeric carbohydrate molecules consisting of long chains of monosaccharides bound together by glycosidic linkages. Common examples of carbohydrates include glucose, fructose, glycogen, starch and cellulose.

Quantitative analysis of carbohydrate building blocks is a powerful tool that can be used to evaluate the potential onset of chronic diseases associated with carbohydrate metabolism. For example, in diagnostic medicine, monitoring plasma glucose levels is a commonly used biomarker for the prognosis of diabetes.

Glucose Assays

Glucose, a monosaccharide, is the most important and widely used carbohydrate in biology. It serves as a ubiquitous energy source for most organisms and is a precursor for the synthesis of several other molecules, such as starch, cellulose and glycogen. The detection of glucose levels in biological samples serves as a key diagnostic parameter for evaluating many metabolic disorders.

Amplite™ Colorimetric Glucose Quantitation assay offers a rapid and robust method for the direct measurement of glucose in biological samples such as cell lysates, biological fluids and growth medium. In this enzyme-coupled assay, glucose is oxidized by glucose oxidase to yield H_2O_2. The level of H_2O_2 produced is measured using our Amplite™ Red peroxidase substrate, which generates a signal proportional to the concentration of glucose in the sample. This assay can detect as little as 3 µM of glucose using an absorbance microplate reader at 570 ± 5 nm. Since this assay is continuous and requires no separation step, it can be readily adapted for automation. Our Amplite™ Fluorimetric Glucose quantitation assay utilizes a similar approach with the capacity to detect as little as 0.1 µM of glucose using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Glucose 6-Phosphate (G6P) Assays

Glucose 6-phosphate (G6P) is a key metabolic intermediate for glucose to penetrate cells, and either to enter a metabolic pathway or to be stored. In cells, G6P is synthesized when glucose is phosphorylated by enzymes such as hexokinase and glucokinase. G6P is also produced during glycogenolysis from glucose 1-phosphate. It is utilized in both glycolysis and the pentose phosphate shunt, or it can be converted to glycogen or starch and stored in hepatocytes and myocytes. G6P is also used by glucose 6-phosphate dehydrogenase (G6PD) to generate equivalents in the form of NADPH. This is particularly crucial in red blood cells where G6PD deficiency leads to hemolytic anemia in states of oxidative stress.

Amplite™ Colorimetric Glucose 6-Phosphaste assay offers a rapid and sensitive method for the quantification of glucose 6-phosphate in biological samples such as cell lysates, biological fluids and growth medium. In this assay, G6P concentration is determined by an enzyme-coupled assay to yield NADPH. The level of NADPH produced is measured using our chromogenic NADPH sensor, which generates a signal proportional to the concentration of G6P in the sample. This assay can detect as little as 1 µM of G6P using an absorbance microplate reader at the ratio of $A_{575\ nm}/A_{605\ nm}$. Our Amplite™ Fluorimetric Glucose quantitation assay utilizes a similar approach with the capacity to detect as little as 0.3 µM of G6P using a fluorescence microplate reader at Ex/Em = 540/590 nm.

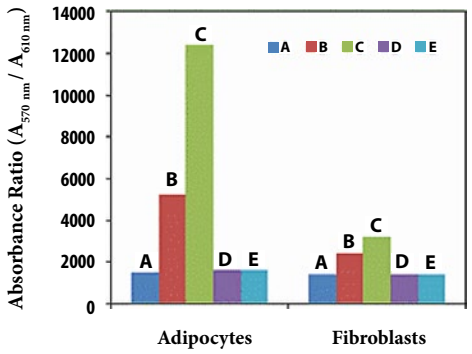

Figure 4.5 Measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Fluorimetric Glucose Uptake Assay Kit (Cat#36500) in a black wall/clear bottom cell culture Poly-D lysine plate using a Gemini (Molecular Devices) microplate reader. (A: Negative Control, no insulin no 2-DG treatment. B: 2DG uptake in the absence of insulin. C: 2DG uptake in the presence of 1µM insulin. D: 2DG uptake in the presence of 1µM insulin and 200 µM phloretin. E: 2DG uptake in the presence of insulin 1µM and 5mM DGlucose.) (Please refer to the protocol for detailed operations.)

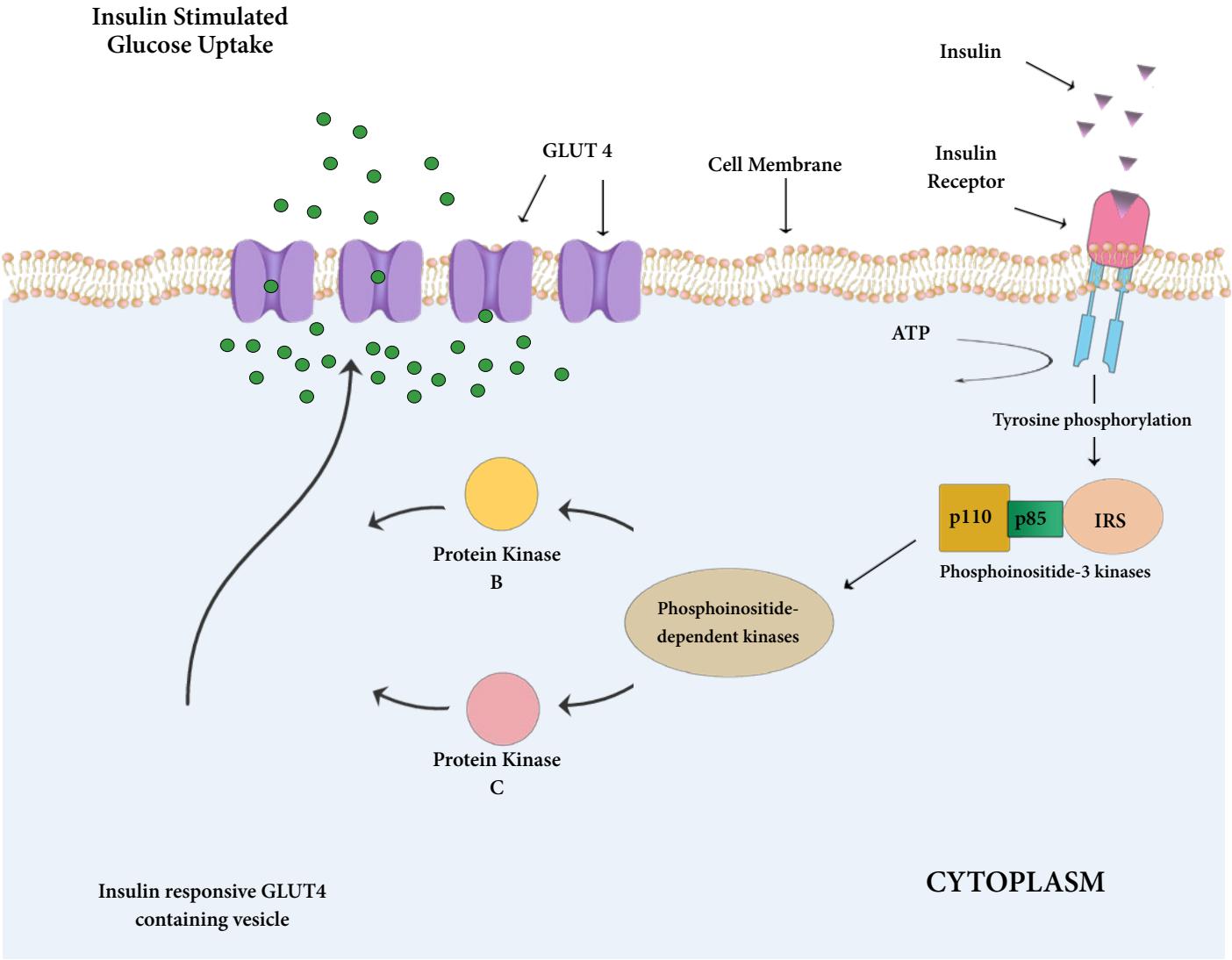

Figure 4.6 Insulin stimulated glucose transportation into cells via glucose transporters GLUT4.

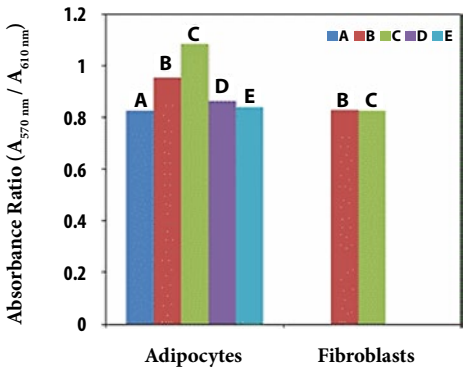

Figure 4.7 Measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Colorimetric Glucose Uptake Assay Kit (Cat#36503) in a black wall/clear bottom cell culture Poly-D lysine plate using a SpectraMax (Molecular Devices) microplate reader. (A: Negative Control, no insulin no 2-DG treatment. B: 2DG uptake in the absence of insulin. C: 2DG uptake in the presence of 1μM insulin. D: 2DG uptake in the presence of 1μM insulin and 200 μM phloretin. E: 2DG uptake in the presence of insulin 1μM and 5mM D-Glucose.) (Please refer to the protocol for detailed operations.)

Glucose Uptake Assays

Glucose metabolism is a key process in many organisms as a primary source of energy. Since its large size prevents simple diffusion across cell membranes, glucose requires the action of specific transmembrane proteins for transport into cells. The rate at which glucose enters cells is tightly regulated by hormones such as insulin, and making it a useful biomarker of metabolic diseases. For example, a lack of insulin-stimulated glucose uptake is linked with type 2 diabetes, while increased glucose uptake is a sign of the high glycolytic rates associated with cancers.

Screen Quest™ Colorimetric Glucose Uptake assay offers a sensitive and nonradioactive assay for measuring glucose uptake in cultured cells. This assay utilizes the glucose analog 2-deoxyglucose (2-DG) which is taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). Since 2-DG6P is non-metabolizable, it accumulates within the cells and the amount accumulated is proportional to glucose uptake. Accumulated 2-DG6P is further oxidized to yield NADPH. The level of NADPH produced is measured using our NADPH sensor, which generates a signal proportional to glucose uptake by cells. The signal can be monitored using an absorbance microplate reader at the ratio of $A_{570\ nm}/A_{610\ nm}$. Our Screen Quest™ Fluorimetric Glucose Uptake assay utilizes a similar approach for measuring glucose uptake by cells and the signal is monitored using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Product Ordering Information For Quantifying Carbohydrate Metabolism

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

Cell Metabolisms

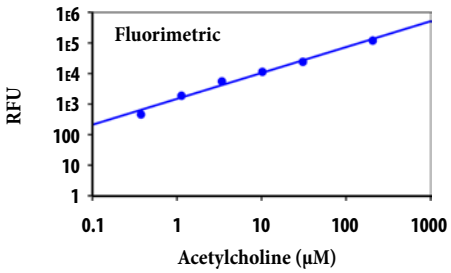

Figure 5.1 Acetylcholine dose response was measured in a 96-well solid black plate with Amplite™ Fluorimetric Acetylcholine Assay Kit (Cat# 11403) using a Gemini fluorescence microplate reader (Molecular devices).

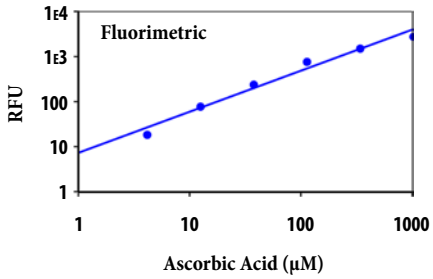

Figure 5.2 Ascorbic Acid dose response was measured with the Amplite™ Fluorimetric Ascorbic Acid Assay Kit (Cat#13835) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 1 µM ascorbic acid can be detected with 30 minutes incubation.

Communication is a complex and dynamic process that continuously occurs within every living organism. Each level of organization-cells, tissues and organs-utilizes numerous signaling pathways and molecules to tightly regulate processes vital for their development, maintenance, and destruction. In multicellular organisms, various types of molecules have the capacity to transmit information between cells. Common examples of signaling molecules include ions, hormones, neurotransmitters, growth factors, cytokines and cell metabolites.

Acetylcholine Assays

Acetylcholine (ACh) and its metabolites are key components utilized in three major physiological processes. It is used in structural integrity and signaling roles for cell membranes, in cholinergic neurotransmission (acetylcholine synthesis), and as a primary source for methyl groups. Acetylcholine is a neurotransmitter used by the central and peripheral nervous systems. In the autonomic division of the peripheral nervous system, it is used as both an internal transmitter for the sympathetic nervous system and the end product released by the parasympathetic nervous system. Acetylcholine disorders have a significant impact on neurological function and have been implicated in the onset and progression of many diseases including diabetic vasculopathy, hypertension and Alzheimer's disease.

Amplite™ Fluorimetric Acetylcholine assay provides an ultrasensitive method for the determination of acetylcholine in solutions and in cell extracts. This assay employs our Amplite™ Red probe to quantify acetylcholine via the choline oxidase-mediated enzyme coupling reaction. The fluorescence intensity generated by Amplite™ Red is proportional to the concentration of acetylcholine in the sample. This assay can detect as little as 0.1 µM of acetylcholine using either an absorbance microplate reader at 575 nm or with a fluorescence microplate reader at Ex/Em = 540/590 nm. Since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Ascorbic Acid Assays

Ascorbic acid, also known as vitamin C, is a critical cell metabolite utilized in many biological processes. It is an essential nutrient involved in tissue growth and repair. It is required for the enzymatic production of neurotransmitters and is important for immune system functionality. The anion of ascorbic acid, ascorbate, acts as a powerful reducing agent in animals and plants rapidly scavenging harmful reactive oxygen species. An antioxidant ascorbate can reduce the risk of chronically developing diseases such as cancer and cardiovascular diseases.

Amplite™ Fluorimetric Ascorbic Acid assay offers a simple and sensitive method for quantifying the total ascorbic acid, as well as, the ratio of dehydroascorbic acid (DHA) to ascorbic acid in biological samples. In this enzyme-coupled assay, ascorbic acid is oxidized to DHA. The level of DHA produced is measured using our Ascorbrite™ Blue fluorescent sensor, which generates a signal proportional to the concentration

of ascorbic acid. This assay can detect as little as 1 µM of total ascorbic acid using a fluorescence microplate reader at Ex/Em = 340/430 nm.

Ammonia Assays

Ammonia is an important source of nitrogen for biological systems. It is required for amino acid synthesis and in animals it is essential for acid/base balance. Ammonia is biosynthesized via amino acid metabolism and is toxic at high concentrations. Hepatocytes help manage ammonia levels by converting it to urea, which is less toxic and less basic. Elevated levels of ammonia in the blood (hyperammonemia) due to defects in enzymes of the urea cycle have been linked to liver dysfunction, such as cirrhosis.

Amplite™ Colorimetric Ammonia Quantitation assay provides a rapid, simple and robust method for the determination of ammonia/ammonium concentration in biological samples such as serum, plasma, and urine. This assay utilizes our proprietary ammonia sensor, which upon reacting with ammonia, yields a blue product. The intensity of the colored reaction product is proportional to the concentration of ammonia in the sample. This assay can detect as little as 4 µM of ammonia using an absorbance microplate reader at 665 nm.

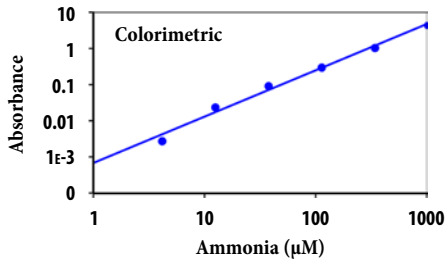

Figure 5.3 Ammonium chloride dose response was measured with Amplite™ Colorimetric Ammonia/Ammonium Quantitation Kit (Cat#10059) in a 96-well clear bottom plate using a Spectrum Max microplate reader (Molecular Devices). As low as 4 µM ammonia can be detected (n=3) in 45 minutes incubation after Assay Buffer II is added.

β-Hydroxybutyrate Assays

β-Hydroxybutyrate (β-HB) is one of three ketone bodies produced in the liver. It is synthesized from the oxidation of fatty acids and transported to peripheral tissues to be used as a source of energy. Ketosis, which is the metabolic state characterized by elevated levels of ketone bodies can occur under normal or pathologic conditions. Normally, ketosis is common under circumstances such as during fasting and prolonged exercise. Pathological causes of ketosis include diabetes, childhood hypoglycemia, and metabolic acidosis (ketoacidosis). In clinical biology, monitoring β-HB is a useful biomarker for diagnosing and monitoring treatment efficacy of ketoacidosis.

Amplite™ Colorimetric β-Hydroxybutyrate assay offers a sensitive and reproducible method for the determination of β-HB concentration in biological samples. In this enzyme-coupled assay, β-HB is oxidized using dehydrogenases. Accompanying this oxidation reaction is the simultaneous reduction of cofactor NAD^+ to NADH. The level of NADH generated is monitored using our NADH sensor, which produces a signal proportional to the concentration of β-HB in the sample. This assay can detect as little as 4 µM of β-HB using an absorbance microplate reader at the ratio of $A_{570\ nm}/A_{610\ nm}$. Our Amplite™ Fluorimetric β-Hydroxybutyrate assay utilizes a similar approach with the capacity to detect as little as 1.4 µM of β-HB using a fluorescence microplate reader at Ex/Em = 540/590 nm.

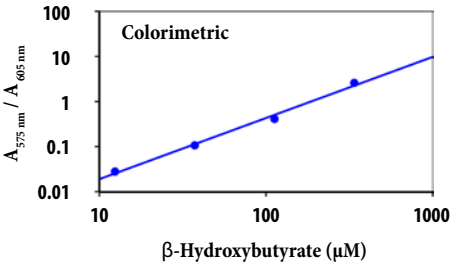

Figure 5.4 β-Hydroxybutyrate (β-HB) dose response was measured with the Colorimetric β-Hydroxybutyrate Assay Kit (Cat#13830) on a black wall/clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 4 µM β-HB can be detected with 30 minutes incubation.

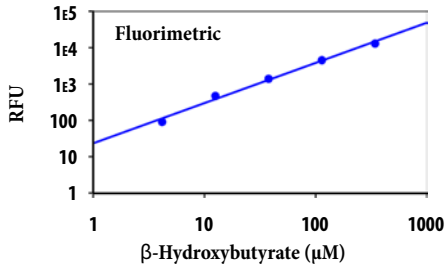

Figure 5.5 β-Hydroxybutyrate (β-HB) dose response was measured with the Fluorimetric β-Hydroxybutyrate Assay Kit (Cat#13831) on a solid black 96-well plate using a Gemini microplate reader. As low as 1.4 µM β-HB can be detected with 10-30 minutes incubation.

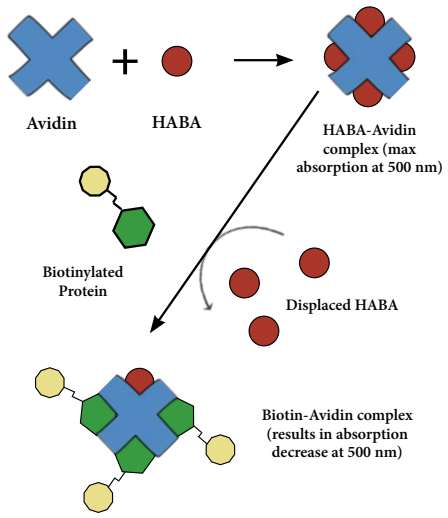

Figure 5.6 HABA assay principle for quantifying biotinylation degree.

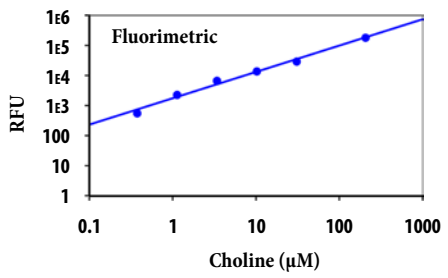

Figure 5.7 Choline dose response was obtained with Amplite™ Choline Quantitation Kit (Cat#40007) in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 100 nM (10 picomole/well) of choline can be detected with 30 minutes incubation time (n=3).

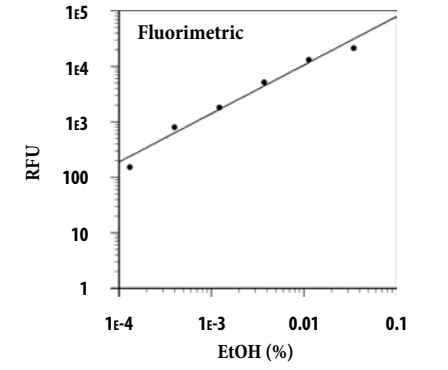

Figure 5.8 Ethanol dose response was measured with Amplite™ Fluorimetric Ethanol Quantitation Kit (Cat#40001) on a 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.0003% of Ethanol was detected with 15 minutes incubation time (n=3).

Biotin Assays

Biotin, also called vitamin H, is a water soluble biomolecule typically utilized in biotinylation reactions for labeling proteins of interest in biochemical assays. Since it's a relatively small molecule, biotin can be conjugated to many proteins, such as antibodies, without altering protein confirmation or biological activity. Its strong affinity for streptavidin molecules (Kd = 10^{-15} M^{-1}) reduces nonspecific binding and significantly improves assay sensitivity. The bond formation between biotin and avidin/streptavidin occurs rapidly, and once formed, it is unaffected by pH, organic solvents and other denaturing agents. Biotinylated protein's tight and specific interaction with avidin/streptavidin has been extensively explored in a number of biological applications including streptavidin pull-down assays, ELISA and Western blotting.

Amplite™ Colorimetric Biotin Quantitation assay offers a convenient method for determining the molar ratio of biotin to protein in biotin-protein conjugates or for quantitating biotin concentration in solution. This assay is based on the differential binding of avidin to biotin, and a dye, 4'-hydroxyazobenzene-2-carboxylic acid (HABA), which shows significant spectral changes when bound to avidin. When biotin is added to the sample, it easily displaces HABA from the HABA/avidin complex. This results in a decrease of absorption at 500 nm, which can be quantitatively correlated to the concentration of biotin in the sample. This assay is best used to determine biotin concentration in the range of 2 to 16 µM using an absorbance microplate reader.

Choline Assays

Choline is an essential nutrient that is a precursor for the neurotransmitter acetylcholine and for the phospholipids phosphatidylcholine and sphingomyelin. It is a water soluble amine that is synthesized in the human body and typically grouped with B-complex vitamins. Choline and its metabolites are necessary for cell membrane integrity, cell signaling and cholinergic neurotransmission (choline synthesis). Choline disorders can have a harmful impact on many neurological processes and its deficiency has been implicated in liver-related diseases, atherosclerosis and 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 scarcely studied.

Amplite™ Choline Quantitation assay provides an ultrasensitive method for the determination of choline in solutions and in cell extracts. This assay is based on an enzyme-coupled reaction that measures choline via choline oxidase activity. Choline oxidation yields H_2O_2 which is detected using our Amplite™ Red probe. Upon association with H_2O_2, Amplite™ Red will generate a fluorescence signal. The intensity of this signal is proportional to the concentration of choline in the sample. This assay can detect as little as 100 nM of choline using either an absorbance microplate reader at 575 ± 5 nm or with a fluorescence microplate reader at Ex/Em = 540/590 nm. It is convenient for 96-well or 384-well microtiter plate format and its optimized "mix and read" format is suitable for HTS applications. Since this assay is continuous and

requires no separation step, it can be readily adapted for automation.

Ethanol Assays

Ethanol, also commonly referred to as alcohol, is as a central nervous system depressant that has significant psychoactive effects in sub lethal doses. While ethanol concentration in blood at 0.1% causes intoxication, levels of 0.5% or higher can be fatal. Excessive consumption of alcohol may also lead to the development of various diseases. The quantitative analysis of ethanol has proved useful in many applications such as basic research, drug discovery, and industrial fermentation processes.

Amplite™ Ethanol Quantitation assay offers a non-radioactive method for the determination of ethanol in biological samples. In this enzyme-coupled assay, ethanol is oxidized by alcohol oxidase to yield H_2O_2. The level of H_2O_2 produced is measured using our Amplite™ Red ethanol reagent, which generates a signal proportional to the concentration of ethanol in the sample. This assay can detect as little as 0.0003% of ethanol using either an absorbance microplate reader at 570 nm or a fluorescence microplate reader at Ex/Em = 540/590 nm. Since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Lactic Acid Assays

Lactic acid is a chiral molecule consisting of two optical isomers: L-lactic acid and D-lactic acid. In animals, the enzyme lactate dehydrogenase catalyzes the conversion of pyruvate to lactate during metabolic processes such as fermentation. When studying cellular and animal physiology, monitoring lactate levels can serve as a biomarker for assessing the balance between tissue oxygen demand and utilization. While both lactate stereoisomers are present in blood, D-lactate is only found at 1-5% the concentration of L-lactate. In humans, elevated levels of D-lactate are typically due to bacterial infection or short bowel syndromes. When D-lactate levels become abnormally high, it is typically indicative of severe conditions such as sepsis, ischemia, acidosis and encephalopathy.

Amplite™ Colorimetric L-Lactic Acid assay provides a robust method for the determination of L-lactate in biological samples such as cell lysates, biological fluids and growth medium. In this enzyme-coupled assay, dehydrogenases catalyze the conversion of lactate to pyruvate. Accompanying this oxidation reaction is the simultaneous reduction of cofactor NAD^+ to NADH. The level of NADH is monitored using our NADH sensor, which produces a signal proportional to the concentration of L-lactate in the sample. This assay can detect as little as 4 µM of lactate using an absorbance microplate reader at the ratio of $A_{575\ nm}/A_{605\ nm}$. Our Amplite™ Fluorimetric L-Lactic Acid assay utilizes a similar approach with the capacity to detect as little as 1.4 µM of L-lactate using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Also available are Amplite™ Colorimetric and Amplite™ Fluorimetric D-Lactic Acid assays for the quantitative analysis of D-lactate in biological samples. They offer the same level of sensitivity and utilize similar methods of determination as the

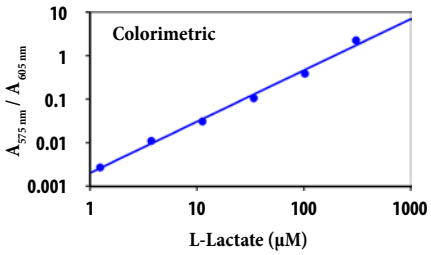

Figure 5.9 L-Lactate dose response was measured with Amplite™ Colorimetric L-Lactate Assay Kit (Cat#13815) in a 96-well white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 4 µM L-Lactate in 100 µL volume can be detected with 1 hour incubation.

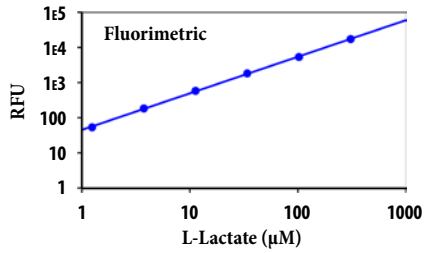

Figure 5.10 L-lactate dose response was measured with Amplite™ Fluorimetric L-lactate Assay Kit (Cat#13814) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1.4 µM L-lactate in 100 µL volume can be detected with 1 hour incubation.

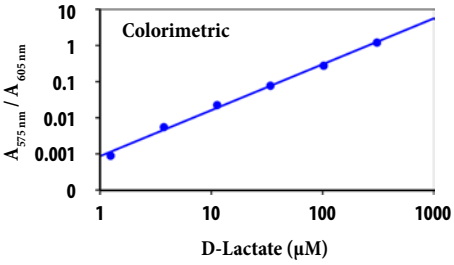

Figure 5.11 D-lactate dose response was measured with Amplite™ Colorimetric D-lactate Assay Kit (Cat#13811) in a 96-well white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 4 µM D-lactate in 100 µL volume can be detected with 1 hour incubation.

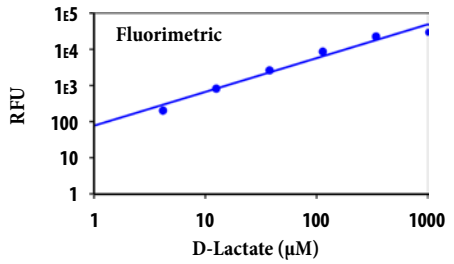

Figure 5.12 D-lactate dose response was measured with Amplite™ Fluorimetric D-Lactate Assay Kit (Cat#13810) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1.4 µM D-lactate in 100 µL volume can be detected with 1 hour incubation.

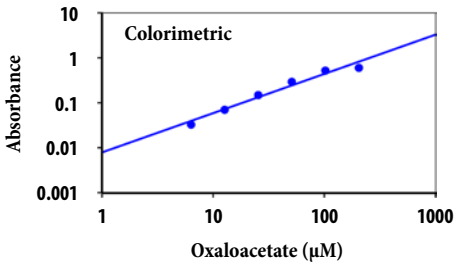

Figure 5.13 Oxaloacetate dose response was measured with the Amplite™ Colorimetric Oxaloacetate Assay Kit (Cat#13840) on a clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 5 µM oxaloacetate can be detected with 30 minutes incubation

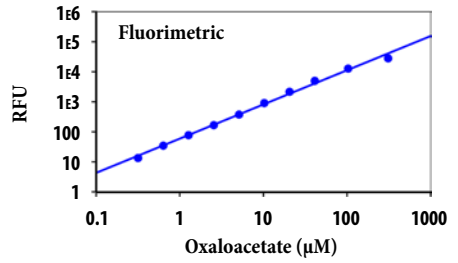

Figure 5.14 Oxaloacetate dose response was measured with Amplite™ Fluorimetric Oxaloacetate Assay Kit (Cat#13841) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 µM oxaloacetate can be detected with 30 minutes incubation

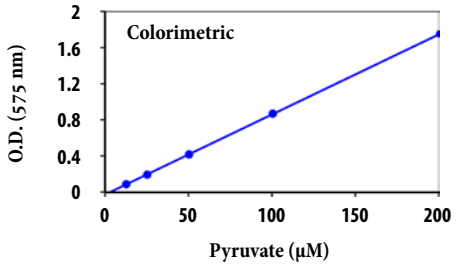

Figure 5.15 Pyruvate dose response was measured with the Amplite™ Colorimetric Pyruvate Assay Kit (Cat#13821) on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 6 µM of pyruvate can be detected with 30 minutes incubation.

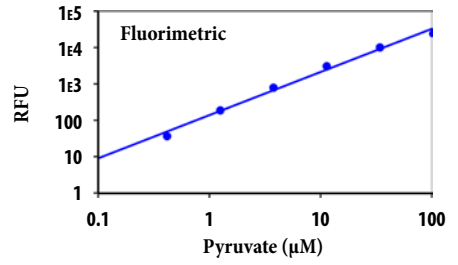

Figure 5.16 Pyruvate dose response was measured with the Amplite™ Fluorimetric Pyruvate Assay Kit (Cat#13820) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 µM pyruvate can be detected with 30 minutes incubation.

aforementioned L-lactate assays.

Oxaloacetate Assays

Oxaloacetate is key metabolic intermediate used in many cellular processes. It is utilized in gluconeogenesis, amino acid synthesis, fatty acid synthesis, the citric acid cycle, urea cycle and glyoxylate cycle. In the citric acid cycle, oxaloacetate reacts with acetyl-CoA to yield citrate. In gluconeogenesis, pyruvate molecules present in the mitochondria are carboxylated to oxaloacetate. This facilitates its transport from the mitochondrial matrix to the cytosol for further downstream synthesis of glucose. Oxaloacetate can be also used as blood glutamate scavengers to provide neuroprotection after traumatic brain injury, expressed both by reduced neuronal loss in the hippocampus and improved neurologic outcomes.

Amplite™ Colorimetric Oxaloacetate assay provides a sensitive method for the determination of oxaloacetate in biological samples. In this enzyme-coupled assay, pyruvate is converted to oxaloacetate which yields a byproduct of H_2O_2. The level of H_2O_2 generated is monitored using our Amplite™ Red substrate, which produces a signal proportional to the concentration of oxaloacetate in the sample. This assay can detect as little as 5 µM of oxaloacetate using an absorbance microplate reader at 575 nm. Our Amplite™ Fluorimetric Oxaloacetate assay utilizes a similar approach with the capacity to detect as little as 0.3 µM of L-lactate using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Pyruvate Assays

Pyruvate is the conjugate base of pyruvic acid and a key intermediate used in several metabolic processes. During the metabolic pathway of glycolysis, one molecule of glucose is converted into two molecules of pyruvate. Living cells utilize pyruvate to provide energy in one of two ways. When oxygen is present (aerobic respiration), pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase which enters the citric acid cycle for ATP generation. When levels of oxygen are insufficient, pyruvate is broken down anaerobically, creating lactate in animals and ethanol in plants and microorganisms. In clinical biology, pyruvate is a useful diagnostic marker for the assessment of metabolic disorders and liver disease.

Amplite™ Colorimetric Pyruvate assay offers a robust method for the quantitative analysis of pyruvate in biological samples. In this enzyme-coupled assay, pyruvate is oxidized by pyruvate oxidase to yield H_2O_2. The level of z produced is measured using our Quest Fluor™ Pyruvate sensor, which generates a signal proportional to the concentration of pyruvate in the sample. This assay can detect as little as 6 µM of pyruvate using an absorbance microplate reader at 575 nm. Our Amplite™ Fluorimetric Pyruvate assay utilizes a similar approach with the capacity to detect as little as 0.3 µM of pyruvate using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Urea Assays

Urea is essential for the metabolism of nitrogen-containing compounds by animals. It is synthesized in the liver as part of the urea cycle either from oxidation of amino acids or from ammonia. Urea is then dissolved in blood and transported to the kidneys to be excreted through urine. In clinical laboratory studies, the determination of urea is powerful tool for monitoring health status. For example, the Blood Urea Nitrogen (BUN) test is a measure the concentration of urea nitrogen levels present in blood. It is primarily used in conjunction with creatinine testing to evaluate kidney function and diagnose kidney related diseases.

Amplite™ Colorimetric Urea Quantitation assay offers a simple and robust method for the determination of urea in biological samples such as serum, plasma and urine. In this enzyme-coupled assay, urea reacts with an enzyme mixture to yield a blue colored product. The color intensity generated is proportional to the concentration of urea in the sample. This assay can detect as little as 10 µM of urea using an absorbance microplate reader at 675 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

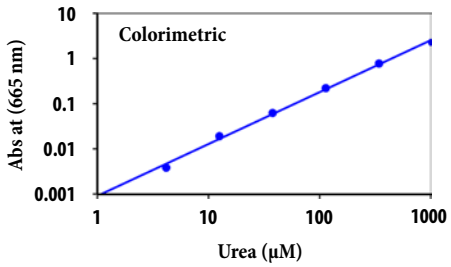

Figure 5.17 Urea dose response in a 96-well clear bottom plate using a Spectrum Max microplate reader (Molecular Devices) measured with Amplite™ Colorimetric Urea Assay Kit (Cat#10058). As low as 10 µM urea can be detected (n=3) in 15 minutes incubation after Assay Buffer II is added.

Xanthine Assays

Xanthine is a purine base found in most body tissues and fluids, and is synthesized from the oxidation of hypoxanthine by xanthine oxidoreductase. It is a precursor for a number of stimulants including caffeine, aminophylline, IBMX, paraxanthine, theobromine, and theophylline. At high concentrations, these stimulants are known to not only activate the respiratory center but stimulate heart rate, force of contraction and cardiac arrhythmias.

Amplite™ Colorimetric Xanthine assay provides a rapid and ultrasensitive method for the determination of xanthine in biological samples. In this enzyme-coupled assay, xanthine is oxidized by xanthine oxidase to yield uric acid and H_2O_2. The level of H_2O_2 produced is measured using our Amplite™ Red substrate, which generates a signal proportional to the concentration of xanthine in the sample. This assay can detect as little as 1.2 µM of xanthine using an absorbance microplate reader at the ratio of $A_{570\ nm}/A_{610\ nm}$. Our Amplite™ Fluorimetric Pyruvate assay utilizes a similar approach with the capacity to detect as little as 0.14 µM of xanthine using a fluorescence microplate reader at Ex/Em = 540/590 nm. Both assays can be performed in a convenient 96-well or 384-well microtiter-plate format.

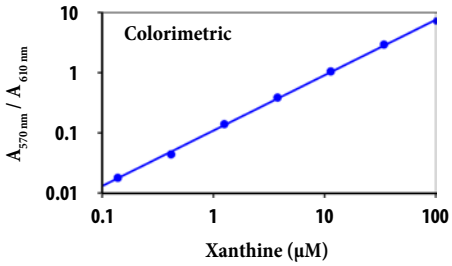

Figure 5.18 Xanthine dose response was measured with Amplite™ Colorimetric Xanthine Assay Kit (Cat#13842) in a 96-well clear bottom plate using a SpectraMax reader (Molecular Devices). As low as 1.2 µM xanthine was detected with 30 minutes incubation time (n=3).

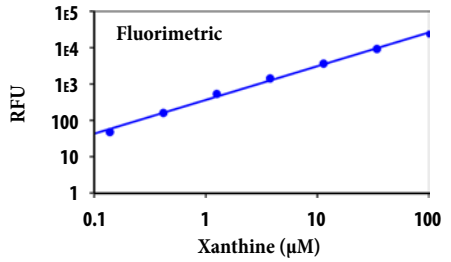

Figure 5.19 Xanthine dose response was measured with Amplite™ Fluorimetric Xanthine Assay Kit (Cat#13843) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.14 µM xanthine was detected with 30 minutes incubation time (n=3).

Product Ordering Information For Quantifying Cell Metaboilsms

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
40007	Amplite™ Choline Quantitation Kit	200 Tests	571	585
10059	Amplite™ Colorimetric Ammonia Quantitation Kit *Blue Color*	200 Tests	650	N/A
13830	Amplite™ Colorimetric Beta-Hydroxybutyrate (Ketone Body) Assay Kit	200 Tests	575	N/A
5522	Amplite™ Colorimetric Biotin Quantitation Kit	200 Tests	500	N/A
13811	Amplite™ Colorimetric D-Lactate Assay Kit	200 Tests	575	N/A
13815	Amplite™ Colorimetric L-Lactate Assay Kit	200 Tests	575	N/A
13840	Amplite™ Colorimetric Oxaloacetate Assay Kit *Red Color*	200 Tests	575	N/A
13821	Amplite™ Colorimetric Pyruvate Assay Kit	200 Tests	575	N/A
10058	Amplite™ Colorimetric Urea Quantitation Kit *Blue Color*	200 Tests	650	N/A
13842	Amplite™ Colorimetric Xanthine Assay Kit	200 Tests	575	N/A
40001	Amplite™ Ethanol Quantitation Kit	200 Tests	571	585
11403	Amplite™ Fluorimetric Acetylcholine Assay Kit *Red Fluorescence*	200 Tests	571	585
13835	Amplite™ Fluorimetric Ascorbic Acid Assay Kit	200 Tests	340	430
13831	Amplite™ Fluorimetric Beta-Hydroxybutyrate (Ketone Body) Assay Kit	200 Tests	571	585
13810	Amplite™ Fluorimetric D-Lactate Assay Kit	200 Tests	571	585
13814	Amplite™ Fluorimetric L-Lactate Assay Kit	200 Tests	571	585
13841	Amplite™ Fluorimetric Oxaloacetate Assay Kit *Red Fluorescence*	200 Tests	571	585
13820	Amplite™ Fluorimetric Pyruvate Assay Kit	200 Tests	571	585
13843	Amplite™ Fluorimetric Xanthine Assay Kit	200 Tests	571	585
5521	ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*	1 kit	N/A	N/A

Cell Signaling

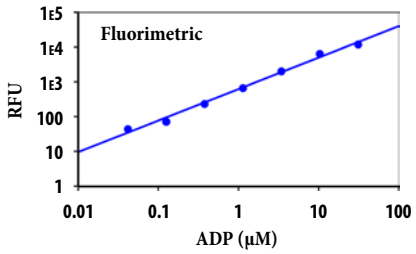

Figure 6.1 ADP dose response was measured with the PhosphoWorks™ Fluorimetric ADP Assay Kit (Cat#21655) in a solid black 384-well plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 μM ADP can be detected with 15, 30 minutes and 1 hour incubation (Z' factor =0.65).

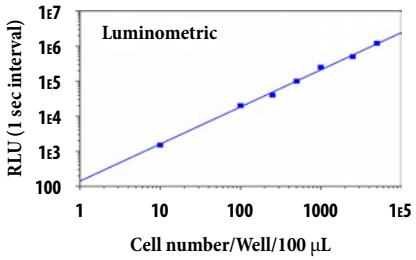

Figure 6.2 CHO-K1 cell number was measured with the PhosphoWorks™ Luminescence ATP Assay Kit (Cat#21610) on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect as low as 10 cells/well (Z' factor= 0.6). The integration time was 1 sec.

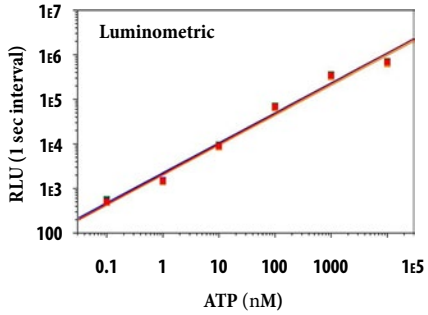

Figure 6.3 ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit (Cat#21609) on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The linear luminescence signal for ATP concentrations from 100 μM to 0.1 nM was monitored for up to 5 hours (Z' factor = 0.7) without signal decay (above figure shows 20 minutes, 1, 2, 3, 4, and 5 hour signal). The integrated time was 1 second.

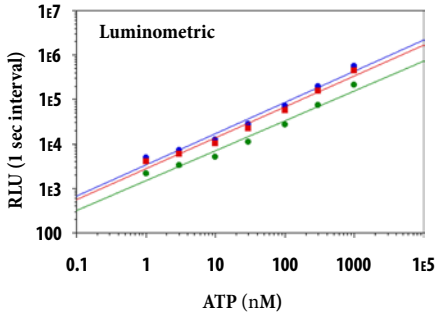

Figure 6.4 ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit (Cat#21610) on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect 3 pmol ATP with 2 hours incubation (Z' factor = 0.6, blue 30 minutes, red 1 hour, and green 2 hours). The integration time was 1 sec. The half life is more than 1.5 hours.

Adenosine Diphosphate Assays

Adenosine diphosphate (ADP) is an important organic molecule that plays a major role in cell metabolism. It is a product of ATP dephosphorylation, a key reaction in living cells that is essential for energy transfer, and can be recycled back to ATP via rephosphorylation. This interconversion of ADP and ATP occurs primarily in the mitochondria and chloroplasts by ATPases such as phosphatases, phosphorylases and kinases. ADP is stored in dense bodies inside blood platelets. When platelets are activated, ADP is released to interact with a family of purinergic receptors thereby regulating cellular function.

PhosphoWorks™ Fluorimetric ADP assay offers a sensitive method for monitoring ADP formation. In this enzyme-coupled assay, ADP formation is measured by monitoring kinase activity due to their proportional relationship. The concentration of ADP produced is directly proportional to enzyme phosphotransferase activity. Kinase activity is monitored using our proprietary ADP sensor and measured fluorimetrically. This assay can detect as little 0.3 μM of ADP using a fluorescence microplate reader at Ex/Em = 540/590 nm. It has a broad ATP tolerance of 1-300 μM making it ideal for determining Michaelis-Menten kinetics, and for screening and identifying kinase inhibitors. It is convenient for 96-well or 384-well microtiter plate format and its optimized "mix and read" format is suitable for HTS applications. Since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Adenosine Triphosphate Assays

Adenosine triphosphate (ATP) is a complex organic molecule that participates in cellular energenics, metabolic regulation and cellular signaling. It is present in all living organisms as the primary energy currency and rapidly degrades in the absence of viable organisms. As such, ATP determination is a useful tool utilized in a variety of research applications to identify the presence of viable organisms. Common applications include cell viability and cytotoxicity, detection of bacterial on surfaces, quantification of bacteria in water, somatic cells in culture, and in food quality and contamination tests.

Our PhosphoWorks™ Luminometric ATP assays provide a fast, simple and homogenous bioluminescence method that can be used for the determination of cell viability, cytotoxicity and proliferation in mammalian cells by ATP detection. The kits employ two components: firefly luciferase and its substrate luciferin. Firefly luciferase is an enzyme that catalyzes the two-step oxidation of luciferin. In the presence of magnesium, luciferase catalyzes the reaction of luciferin, ATP and oxygen to yield an emission of light at 560 nm. When ATP is the limiting component, the intensity of light is proportional to the concentration of ATP. This assay is highly sensitive with the capacity to detect as little as 3 pmol of ATP using a luminescence microplate reader. It can be performed in a convenient 96- well or 384-well microtiter-plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Cyclic Adenosine Monophosphate Assays

Cyclic Adenosine Monophosphate (cAMP) is a vital second messenger utilized in many biological pathways. It is utilized for intracellular signal transduction in many organisms, conveying the cAMP-dependent pathway, and is involved in the activation of protein kinases and ion channel regulation. cAMP is derived from ATP and synthesized by plasma membrane-bound adenylyl cyclase which are coupled to transmembrane receptors. Activation of adenylyl cyclase is initiated by a range of signaling molecules. These molecules interact with receptors, such as GPCRs, which initiate adenylyl cyclase activity and increase intracellular cAMP levels. Finally, cAMP activates cAMP-dependent proteins kinases that are responsible for phosphorylating specific substrate proteins. Determination of cAMP activity is useful for functional assays assessing receptor activation, receptor characterization, and receptor-ligand identification.

Our Screen Quest™ Colorimetric ELISA cAMP assay functions, in principle, as other direct competitive ELISAs. Anti-cAMP primary antibodies are coated onto the surface of a microplate well. Then a test sample is added; cAMP in the test sample will bind to the coated antibodies. Afterwards, cAMP-HRP is introduced, which will displace the bound cAMP due to the antibodies' higher affinity for cAMP-HRP over unmodified cAMP. Finally, a chromogenic reagent is added and oxidized by the bound cAMP-HRP, generating a signal proportional to the cAMP-HRP concentration and inversely proportional to the cAMP concentration. This assay can detect as little as 0.1 nM of cAMP using an absorbance microplate reader at 405 nm, 650 nm or 740 nm. Our Screen Quest™ Fluorimetric ELSIA cAMP assay utilizes a similar approach using a fluorogenic substrate to detect HRP activity. This assay has the capacity to detect as little as 1 nM of cAMP using a fluorescence microplate reader at Ex/Em = 540/590 nm.

Screen Quest™ TR-FRET No Wash cAMP assay provides a convenient method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. This assay is based on the competition for a fixed number of anti-cAMP antibody binding sites between fluorescent cAMP tracers and non-labeled free cAMP. Free cAMP displaces the fluorescent cAMP tracer from the HRP-cAMP/anti-cAMP antibody complex. To monitor activity anti-cAMP antibodies are labeled with trFluor™ Tb, while cAMP tracers are conjugated with trFluor™ 650. In the absence of cAMP, competition is minimal allowing for the formation of cAMP-trFluor™ 650 and trFluor™-anti-cAMP antibody complexes that generate strong FRET fluorescence. When free cAMP is introduced to the system, it competes for the trFluor™-anti-cAMP antibody conjugate, thereby inhibiting the binding of cAMP-trFluor™ 650 conjugate, resulting in the elimination of fluorescence. The magnitude of FRET is proportional to the concentration of cAMP in a sample. This kit can detect as little as 1 nM of cAMP using a time-resolved fluorimetric microplate reader.

Screen Quest™ Live Cell cAMP Assay Service Pack permits the real-time monitoring of intracellular cAMP change in a high-throughput format without a cell lysis step. The assay is compatible for cell lines that contain either an exogenous cyclic nucleotide-

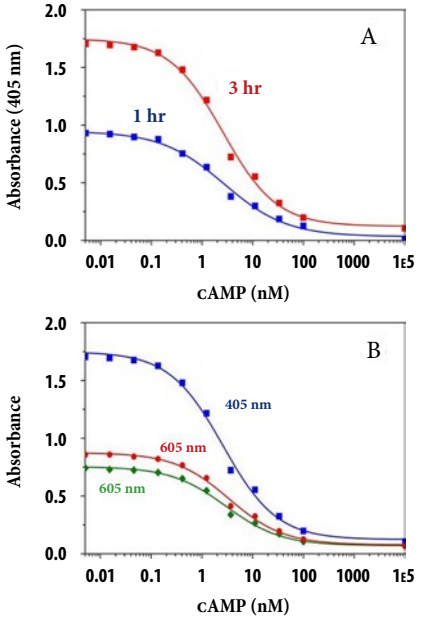

Figure 6.5 cAMP dose response was measured with Screen Quest™ Colorimetric ELISA cAMP Assay Kit (Cat#36370) in a clear 96-well plate with a SpectraMax microplate reader. A: The kit can detect as low as 0.1 nM cAMP in a 100 µL reaction volume at 405nm after incubation with Amplite™ Green for 1 hour (blue line) and 3 hours (red line). B: The Absorbance can be read at 405 nm (blue line), 650 nm (red line) or 740 nm (Green line), the data in figure B are from the incubation with Amplite™ Green for 3 hours.

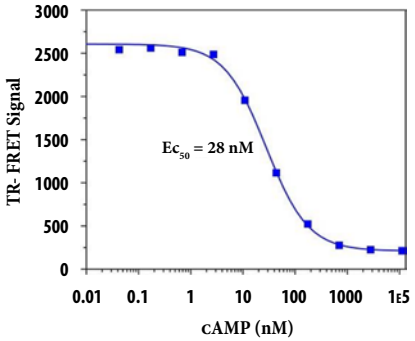

Figure 6.6 cAMP dose response was measured with Screen Quest™ cAMP FRET Assay Kit (Cat#36379) using a ClarioStar microplate reader (BMG). The kit can detect as low as 1 nM cAMP .

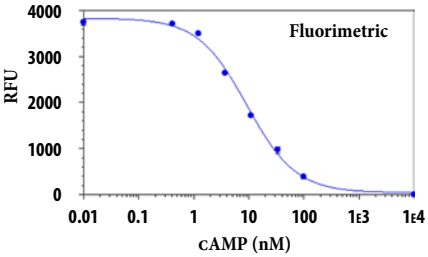

Figure 6.7 cAMP dose response was measured with Screen Quest™ Fluorimetric ELISA cAMP Assay Kit (Cat#36373) in a solid black 96-well plate with a Gemini microplate reader. The kit can detect as low as 1 nM cAMP in a 100 µL reaction volume.

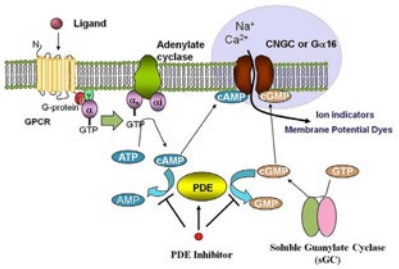

Figure 6.8 Screen Quest™ Live Cell cAMP Assay (Cat#36382) Principle.

gated channel (CNGC) or the promiscuous G-protein, Gα16. Channel activation is characterized by elevated levels of intracellular cAMP that result in ion flux and cell membrane depolarization, both of which are detectable using fluorescent ion indicators such as Cal-520® AM. Co-expression of Gα16 with specific non-Gq-coupled receptors also results in the generation of an intracellular calcium signal upon receptor stimulation. This assay includes both cell lines and reagents for the measurement of intracellular cAMP changes with a FLIPR, a FDSS or other equivalent fluorescence microplate reader.

Product Ordering Information For Quantifying Cell Signaling Molecules

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
31001	Amplite™ Universal Fluorimetric Kinase Assay Kit *Red Fluorescence*	250 Tests	571	585
31002	Amplite™ Universal Fluorimetric Kinase Assay Kit *Red Fluorescence*	500 Tests	571	585
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	100 Tests	571	585
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 Plate	N/A	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	1 Plate	N/A	560
21613	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	10 Plates	N/A	560
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	1 Plate	N/A	560
36370	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	1 plate	650	N/A
36371	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	10 plates	650	N/A
36373	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	1 plate	571	585
36374	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	10 plates	571	585
36379	Screen Quest™ FRET No Wash cAMP Assay Kit	1 plate	390	650
36380	Screen Quest™ FRET No Wash cAMP Assay Kit	10 plates	390	650
36381	Screen Quest™ FRET No Wash cAMP Assay Kit	50 plates	390	650
36382	Screen Quest™ Live Cell cAMP Assay Service Pack	Each	490	520

Enzyme Cofactors

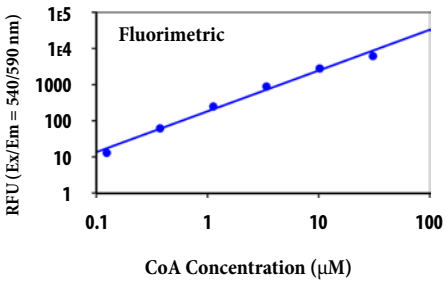

Figure 7.1 CoA dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Coenzyme A Quantitation Assay Kit (Cat#15270) using a NOVOstar microplate reader (BMG Labtech). As low as 40 nM (4 pmol/well) of CoA was detected with 30 minutes incubation time (n=3).

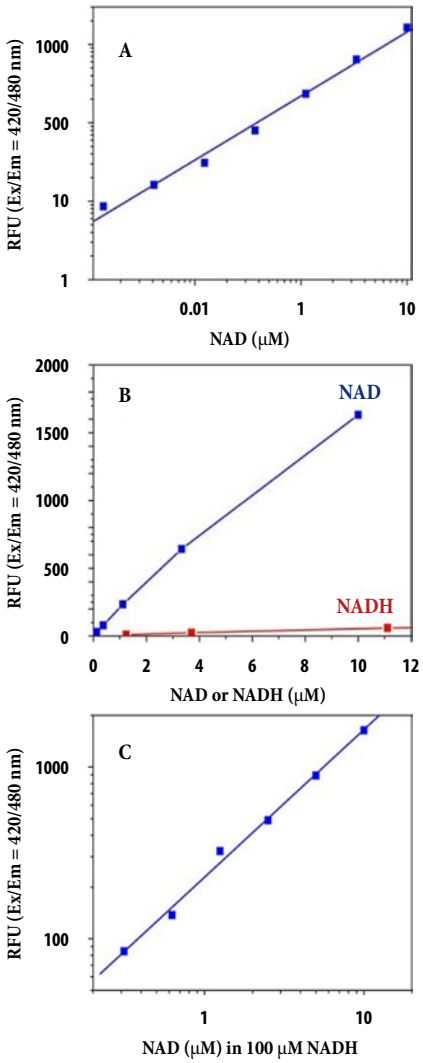

Figure 7.2 NAD dose response was measured with Amplite™ Fluorimetric NAD Assay Kit (Cat#15280) in a 96-well black/solid bottom plate using a Germini microplate reader (Molecular devices). A: NAD standard curve, as low as 30 nM of NAD can be detected with 20 min incubation (n=3). B: Comparison of NAD and NADH response C: NAD standard curve with 100 μM NADH in presence in the solution. As low as 0.3% of NAD (~300 nM) converted from NADH can be detected with 20 min incubation (n=3).

Coenzyme A Assays

Coenzyme A (CoA) is an essential cofactor present in all cellular organisms. It is comprised of units derived from cysteine, pantothenic acid and adenosine triphosphate. Coenzyme A is a principal acyl carrier in many biosynthetic, energy-yielding, and degradative pathways. It plays a key role in the synthesis and oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle. Coenzyme A levels can be altered during starvation, and in conditions such as cancer, diabetes and alcoholism.

Amplite™ Fluorimetric Coenzyme A Quantitation assay offers an ultrasensitive method for the determination of coenzyme A in biological samples. This assay utilizes our fluorogenic sensor CoA Green™ to detect the thiol (-SH) groups of coenzyme A. Upon association with thiol groups, CoA Green™ generates a strong fluorescence signal. The fluorescence intensity of the signal is directly proportional to the concentration of coenzyme A in solution. This assay can detect as little as 40 nM of coenzyme A using a fluorescence microplate reader at Ex/Em = 490/520 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

NAD/NADH and NADP/NADPH Detection

Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are key cofactors found in all living cells. NAD^+ is present in two forms: an oxidized (NAD^+) and reduced (NADH) form. It is utilized primarily in the redox reactions of metabolic processes as an electron carrier. NAD^+ also forms $NADP^+$ by the addition of a phosphate group ester linked to the 2' position of the adenyl nucleotide. The reduced from of $NADP^+$ is NADPH. Organisms use $NADP^+$ in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which requires NADPH as a reducing agent. Chloroplasts utilize $NADP^+$ as an oxidizing agent essential in the preliminary reactions of photosynthesis. The NADPH produced during photosynthesis is subsequently utilized for its reducing capabilities by biosynthetic reactions in the Calvin cycle of photosynthesis.

Traditional assays for the determination of NAD^+/NADH and $NADP^+$/NADPH are based on monitoring changes in absorption of NADH or NADPH at the wavelength of 340 nm. However, when working in this short UV wavelength range, assays are less sensitive as a result of high levels of interference from autofluorescing proteins. To remedy this, AAT Bioquest has developed NAD^+/NADH and $NADP^+$/NADPH determination assays that operate in the visible wavelength range where interference is minimal, resulting in assays with wide dynamic ranges and significantly improved sensitivity. This section describes our comprehensive portfolio of products for the determination of NAD^+/NADH and $NADP^+$/NADPH.

NAD Assays

Amplite™ Fluorimetric NAD assay provides a sensitive and rapid detection of NAD^+

in biological samples. This assay utilizes our proprietary Quest Fluor™ NAD probe to directly measure NAD^+. Upon association with NAD^+, Quest Fluor™ NAD generates a fluorescence signal that is measured using a fluorescence microplate reader at Ex/Em = 420/480. This assay has minimal response to NADH and as little as 30 nM NAD^+ can be detected in samples. It is convenient for 96-well or 384-well microtiter plate format and its optimized "mix and read" format is suitable for HTS applications.

NADH Assays

Amplite™ Colorimetric NADH assay offers a simple and robust method for the determination of NADH in biological samples. The NADH probe used in this assay is a chromogenic sensor that has a maximum absorbance at 460 nm upon NADH reduction. This assay can detect as little as 3 µM of NADH in samples using an absorbance microplate reader at 460 nm. The absorbance increase is proportional to the concentration of NADH in the solution.

Our Amplite™ Fluorimetric NADH assay employs a system of enzymes that specifically recognize NADH via an enzyme cycling reaction. It utilizes a proprietary NADH sensor, which upon association with NADH yields a red fluorescent product. The fluorescence intensity generated is proportional to the concentration of NADH in the solution. This assay can detect as little as 1 µM of NADH in samples using either an absorbance microplate reader at 575 nm or a fluorescence microplate reader at Ex/Em = 540/590 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Total NAD and NADH Assays

Amplite™ Colorimetric Total NAD and NADH assay offers a convenient and sensitive method for the determination of NAD^+/NADH in solutions and cell extracts. This assay employs a system of enzymes that specifically recognize NAD^+/NADH via an enzyme cycling reaction. The NAD^+/NADH probe used in this assay 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. This assay can detect as little as 300 nM of NAD^+/NADH in samples using an absorbance microplate reader at 460 nm or at the absorbance ratio of $A_{570\ nm}/A_{605\ nm}$ for improved sensitivity.

Our Amplite™ Fluorimetric Total NAD and NADH assay utilizes a similar system of enzymes that specifically recognize NAD^+/NADH via an enzyme cycling reaction. It utilizes our proprietary NADH sensor, which upon association with NAD^+/NADH yields a red fluorescent product. The fluorescence intensity generated is proportional to the concentration of NAD^+/NADH in the solution. This assay can detect as little as 100 nM of NAD^+/NADH in samples using a fluorescence microplate reader at Ex/Em = 540/590 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

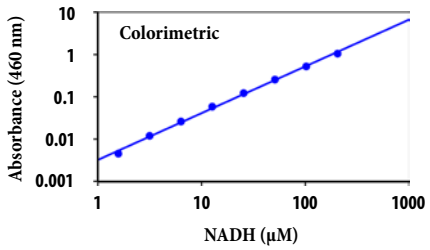

Figure 7.3 NADH dose response was measured with Amplite™ Colorimetric NADH Assay Kit (Cat#15271) in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular devices). As low as 3 µM of NADH can be detected with 30min incubation (n=3) with absorbance measurement at 460 nm.

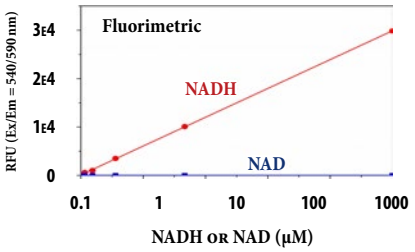

Figure 7.4 NADH dose response was measured with Amplite™ Fluorimetric NADH Assay Kit (Cat#15261) in a 96-well black plate using a NOVOStar microplate reader (BMG Labtech). As low as 1 µM (100 pmols/well) of NADH can be detected with 1 hour incubation (n=3) while there is no response from NAD.

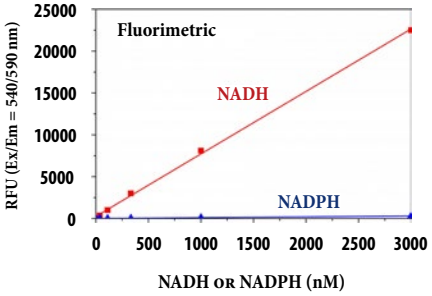

Figure 7.5 NADH dose response was measured with Amplite™ Fluorimetric Total NAD and NADH Assay Kit (Cat#15257) in a solid black 96-well plate using a NOVOStar microplate reader (BMG Labtech). As low as 100 nM (10 pmol/well) of NADH can be detected with 1 hour incubation (n=3) while there is no response from NADPH.

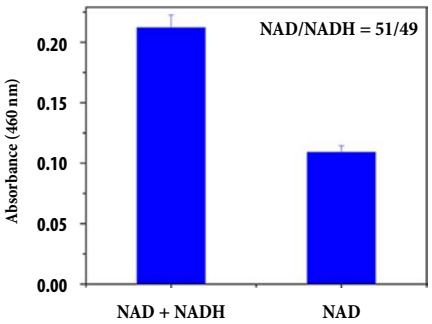

Figure 7.6 Amplite™ Colorimetric NAD/NADH Ratio Assay Kit (Cat#15273) was used to measure NAD/NADH ratio in a 96-well white wall/clear bottom microplate using a SpectraMax® microplate reader (Molecular Devices). Equal amount of NAD and NADH mixtures were treated with or without NAD extraction solution 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.

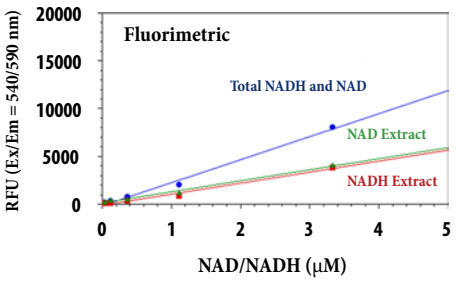

Figure 7.7 Total NADH/NAD, and their extract dose response were measured with Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit (Cat#15263) in a 96-well black plate using a Gemini microplate reader (Molecular Devices). 25 µL of equal amount of NAD and NADH was treated with or without NADH or NAD extraction solution for 15 min, and then neutralized with extraction solutions at room temperature. The signal was read at Ex/Em = 540/590 nm 30 min after adding 75 µL of NADH reaction mixture. The blank signal was subtracted from the values for those wells with the NADH reactions.

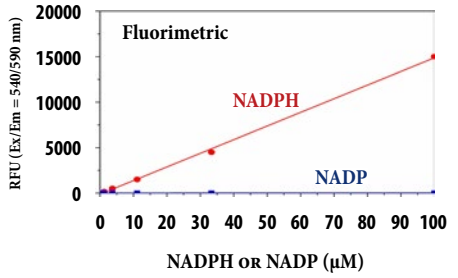

Figure 7.8 NADPH dose response was measured with Amplite™ Fluorimetric NADPH Assay Kit (Cat#15262) in a 96-well black plate using a NOVOStar microplate reader (BMG Labtech). As low as 1 µM of NADPH can be detected with 1 hour incubation (n=3) with no response to NADP.

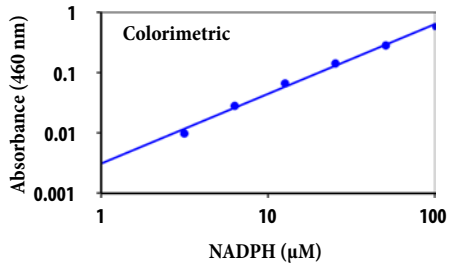

Figure 7.9 NADPH dose response was measured with Amplite™ Colorimetric NADPH Assay Kit (Cat#15272) in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular devices). As low as 3 µM of NAPDH can be detected with 30min incubation (n=3) with absorbance measurement at 460nm.

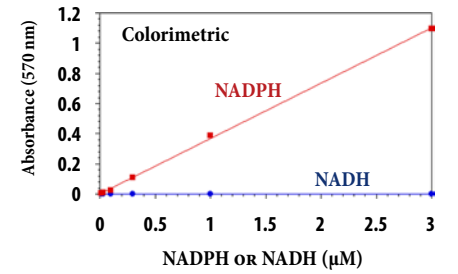

Figure 7.10 NADPH dose response was measured with Amplite™ Colorimetric Total NADP and NADPH Assay Kit (Cat#15260) in a white/clear bottom 96-well plate using a NOVOStar microplate reader (BMG Labtech). As low as 100 nM (10 pmol/well) of NADPH can be detected with 1 hour incubation (n=3) while there is no response from NADH.

NAD/NADH Ratio Assays

NAD^+ and $NADP^+$ function as electron carriers in many redox reactions. The balance between oxidized and reduced forms is referred to as the NAD^+/NADH ($NADP^+$/NADPH) ratio. This ratio is an important component to assess the redox state of a cell, and it is a measurement that reflects both the metabolic activities and the health of cells.

Amplite™ Colorimetric NAD/NADH Ratio assay offers a sensitive method for determining intracellular total NAD^+/NADH amount, as well as, the NAD^+/NADH ratio in culture cells. In this assay, NAD^+ present in cell lysate is extracted using an NAD^+ extraction solution and enzymatically converted to NADH. The level of NADH produced is recognized using or NADH probe to yield a yellow-color dye that is monitored with an absorbance microplate reader at 460 nm. The amount of the dye generated is directly proportional to the concentration of NAD^+/NADH in the cell lysate and can be used as an indicator of the cellular NAD^+/NADH concentration.

Our Amplite™ Fluorimetric NAD^+/ NADH Ratio assay employs a system of enzymes that specifically recognize NAD^+/NADH via an enzyme cycling reaction. Compared with other detection methods, our enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has been shown to have very low background. This is due to its spectral properties, which lie in the red visible range, significantly reducing interference from biological samples. For researchers concerned about usability, our kit is very convenient in its application. There is no need to purify NAD^+/NADH from the sample prior to use; simply mix-and-read using a fluorescence microplate reader at Ex/Em = 540/590 nm. For convenience, this kit includes NAD^+ and NADH extraction buffer as well as a cell lysis buffer.

NADP Assays

Amplite™ Fluorimetric NADP assay provides a sensitive and rapid detection of $NADP^+$ in biological samples. This assay utilizes our proprietary Quest Fluor™ NADP probe to directly measure $NADP^+$ with minimal response to NADPH. Upon association with $NADP^+$, Quest Fluor™ NADP generates a fluorescence signal that is monitored using a fluorescence microplate reader at Ex/Em = 420/480. This assay can detect as little as 30 nM of $NADP^+$, and monitor 0.3% $NADP^+$ generation in the presence of excess NADPH. It is convenient for 96-well or 384-well microtiter plate format and its optimized "mix and read" format is suitable for HTS applications.

NADPH Assays

Amplite™ Colorimetric NADPH assay offers a simple and robust method for the determination of NADPH in biological samples. The NADPH probe used in this assay 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. This assay can detect as little as 3 µM of NADH in samples using an absorbance microplate reader at 460 nm.

Our Amplite™ Fluorimetric NADPH assay employs a system of enzymes that specifically recognize NADPH via an enzyme cycling reaction. It utilizes a proprietary NADPH sensor, which upon association with NADPH yields a red fluorescent product. The fluorescence intensity generated is proportional to the concentration of NADPH in the solution. This assay can detect as little as 1 µM of NADPH in samples using either an absorbance microplate reader at 575 nm or a fluorescence microplate reader at Ex/Em = 540/590 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

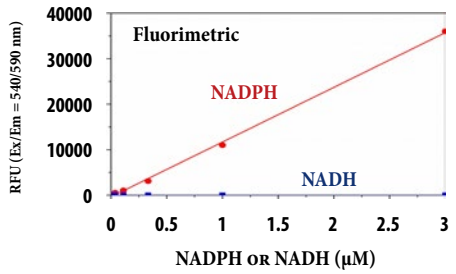

Figure 7.11 NADPH dose response was measured with Amplite™ Fluorimetric total NADP and NADPH Assay Kit (Cat#15259) in a black 96-well plate using a NOVOStar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of NADPH can be detected with 30 minutes incubation (n=3) while there is no response from NADH.

Total NADP and NADPH Assays

Amplite™ Colorimetric Total NADP and NADPH assay offers a convenient and sensitive method for the determination of $NADP^+$/NADPH in solutions and cell extracts. This assay employs a system of enzymes that specifically recognize $NADP^+$/NADPH via an enzyme cycling reaction. The $NADP^+$/NADPH probe used in this assay is a chromogenic sensor that has its maximum absorbance at 575 nm upon $NADP^+$/NADPH reduction. The absorption of the $NADP^+$/NADPH probe is directly proportional to the concentration of $NADP^+$/NADPH. This assay can detect as little as 100 nM of NAD^+/NADH in samples using an absorbance microplate reader at 575 ± 5 nm or at the absorbance ratio of $A_{570\ nm}/A_{605\ nm}$ for improved sensitivity.

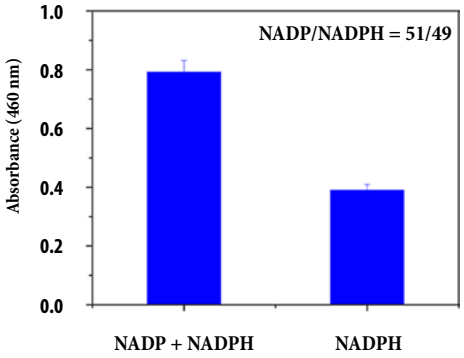

Figure 7.12 Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit (Cat# 15274) was used to measure NADP/NADPH ratio in a 96-well white wall/clear bottom microplate using a SpectraMax® microplate reader (Molecular Devices). Equal amount of NADP and NADPH mixtures were treated with or without NADPH extraction solution for 15 minutes, and then neutralized with extraction solution at room temperature. The sig- nal was read at 460 nm. NADP/NADPH ratio was calculated based on the absorbance shown in the figure.

Our Amplite™ Fluorimetric Total NADP and NADPH assay utilizes a similar system of enzymes that specifically recognize $NADP^+$/NADPH via an enzyme cycling reaction. It utilizes our proprietary NADPH sensor, which upon association with $NADP^+$/NADPH yields a red fluorescent product. The fluorescence intensity generated is proportional to the concentration of $NADP^+$/NADPH in the solution. This assay can detect as little as 10 nM of $NADP^+$/NADPH in samples using a fluorescence microplate reader at Ex/Em = 540/590 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation

NADP/NADPH Ratio Assays

Amplite™ Colorimetric NADP/NADPH Ratio assay offers a sensitive method for determining intracellular total $NADP^+$/NADPH amount, as well as, the $NADP^+$/NADPH ratio in culture cells. In this assay, $NADP^+$ present in cell lysate is extracted using an $NADP^+$ extraction solution and enzymatically converted to NADH. The level of NADH produced is recognized using or NADPH probe to yield a yellow-color dye that is monitored with an absorbance microplate reader at 460 nm. The amount of the dye generated is directly proportional to the concentration of $NADP^+$/NADPH in the cell lysate and can be used as an indicator of the cellular $NADP^+$/NADPH concentration.

Our Amplite™ Fluorimetric NADP/NADPH Ratio assay employs a system of enzymes that specifically recognize $NADP^+$/NADPH via an enzyme cycling reaction. Compared with other detection methods, our enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has been shown to have very

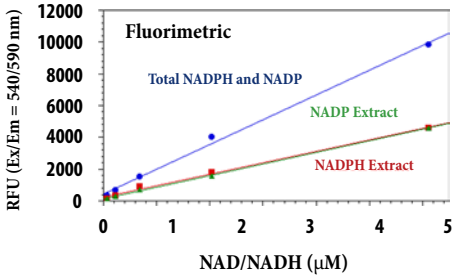

Figure 7.13 Total NADPH and NADP, and their extract dose response were measured with Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit (Cat#15264) in a 96-well black plate using a Gemini microplate reader (Molecular Devices). 25 µL of equal amount of NADP and NADPH was treated with or without NADPH or NADP 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 of NADPH reaction mixture. The blank signal was subtracted from the values for those wells with the NADPH reactions (Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point).

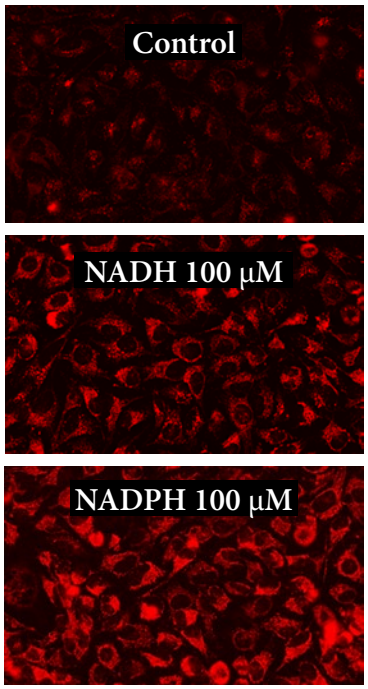

Figure 7.14 Fluorescence images of NADH/NADPH in HeLa cells using Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit (Cat#15290). HeLa cells were incubated with 100 μM NADH or 100 μM NADPH in serum-free medium for 30 minutes and then co-incubated with JZL1707 NAD(P)H sensor working solution for another 30 minutes. The fluorescence signal was measured using fluorescence microscope with a Cy3® filter.

low background. This is due to its spectral properties, which lie in the red visible range, significantly reducing interference from biological samples. For researchers concerned about usability, our kit is very convenient in its application. There is no need to purify $NADP^+$/NADPH from the sample prior to use; simply mix-and-read using a fluorescence microplate reader at Ex/Em = 540/590 nm. For convenience, this kit includes $NADP^+$ and NADPH extraction buffer as well as a cell lysis buffer.

Intracellular NADH/NADPH Detection

Measuring the intracellular concentration of dihydronicotinamide adenine dinucleotide (NADH) and its phosphate ester (NADPH) is a useful tool in disease diagnostic and as a therapeutic target. In organisms, the redox couples of NAD^+/NADH and $NADP^+$/NADPH play key roles in energy metabolism, glycolysis, tricarboxylic acid cycle and mitochondrial respiration. When intracellular $NADP^+$ or NADPH levels elevate, cells are prone to DNA damage resulting from the abnormal production of reactive oxygen species (ROS).

Our Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging assay offers an efficient method for monitoring intracellular $NADP^+$/NADPH levels in live cells. This assay utilizes our cell-permeable JZL1707 NAD(P)H sensor, which binds NADH/NADPH with high sensitivity and specificity. Upon association with NADH/NADPH, JZL1707 NAD(P)H sensor generates a strong fluorescence signal. This signal can either be monitored using a fluorescence microplate reader at Ex/Em = 540/590 nm or visualized using a fluorescence microscope equipped with a Cy3® or TRITC filter set.

Product Ordering Information For Quantifying Enzyme Cofactors

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
15273	Amplite™ Colorimetric NAD/NADH Ratio Assay Kit	250 Tests	460	N/A
15271	Amplite™ Colorimetric NADH Assay Kit	400 Tests	460	N/A
15274	Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit	250 Tests	460	N/A
15272	Amplite™ Colorimetric NADPH Assay Kit	400 Tests	460	N/A
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	460	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	460	N/A
15270	Amplite™ Fluorimetric Coenzyme A Quantitation Kit *Green Fluorescence*	200 Tests	510	524
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
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
15291	Cell Meter™ Intracelluar NADH/NADPH Flow Cytometric Analysis Kit	100 Tests	540	590
15290	Cell Meter™ Intracelluar NADH/NADPH Fluorescence Imaging Kit	100 Tests	540	590
15266	ReadiUse™ NADP Regenerating Kit	200 Tests	N/A	N/A
15265	ReadiUse™ NADPH Regenerating Kit	1000 Tests	N/A	N/A

Oxidative Stress

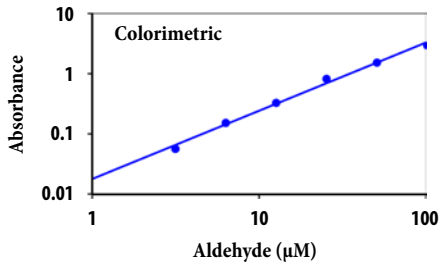

Figure 8.1 Aldehyde dose response was measured in a white wall/clear bottom 96-well plate with Amplite Colorimetric Aldehyde Quantitation Kit (Cat#10053) using a SpectraMax microplate reader (Molecular Devices).

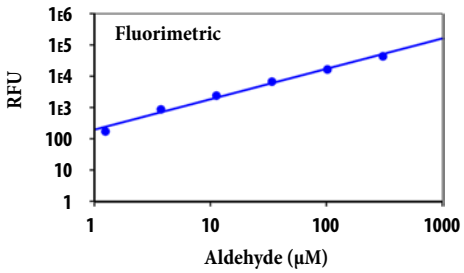

Figure 8.2 Aldehyde dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Aldehyde Quantitation Kit (Cat#10052) using a Gemini fluorescence microplate reader (Molecular Devices).

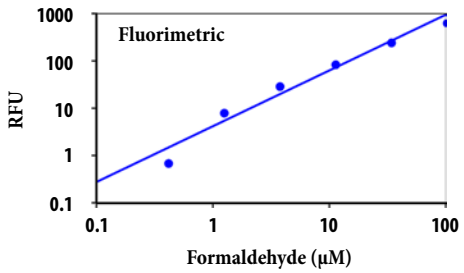

Figure 8.3 Formaldehyde dose response was measured in a 96-well solid black plate with Amplite™ Fluorimetric Formaldehyde Quantitation Kit (Cat#10057) using a Gemini fluorescence microplate reader (Molecular Devices).

Aldehyde Assays

Reactive aldehydes, primarily 4-hydroxyalkenals, were initially shown to be a product of autoxidizing chemical systems. However, it was subsequently demonstrated to be produced in substantial amounts under biological conditions, such as, during lipid peroxidation of liver microsomes or hepatocytes. Additionally, many other aldehydes including alkanals, alk-2-enals, and 4-hydroxyalkenals, were also identified during lipid peroxidation.

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.

Our Amplite™ Colorimetric Aldehyde Quantitation assay offers a rapid and robust method for the determination of aldehydes in biological samples. It utilizes our proprietary aldehyde sensor AldeView™ Yellow, which upon reacting with aldehydes generates a chromogenic product. This assay can detect as little as 10 µM of aldehydes using an absorbance microplate reader to monitor absorbance increase at 405 or 550 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Amplite™ Fluorimetric Aldehyde Quantitation assay offers an ultrasensitive method for the determination of aldehydes in biological samples at higher pH. It utilizes our proprietary fluorogenic sensor AldeLight™ Blue, which yields a strongly fluorescent product upon reacting with aldehydes. The fluorescence intensity of the signal is directly proportional to the concentration of aldehyde in solution. This assay can detect as little as 3 µM of aldehydes using a fluorescence microplate reader at Ex/Em = 365/435 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Formaldehyde Assays

Formaldehyde is a naturally occurring substance and the simplest of all the aldehydes. It is commonly utilized in industrial processes due to its high reactivity with many other chemical compounds. It acts as a disinfectant and biocide and in histological applications as a tissue fixative. Since formaldehyde has the capacity to covalently bind macromolecules, such as DNA, it is a highly toxic compound at abnormal levels. As a pollutant, formaldehyde has been well-identified as volatile chemical contaminant responsible for indoor pollution and "building sick" syndrome disease and it has been recently classified as carcinogenic. Rapid and accurate determination of formaldehyde is an important task for biological research, food industry, chemical

research and environmental pollution surveillance.

Amplite™ Fluorimetric Formaldehyde Quantitation assay provides a simple and sensitive method for the quantification of formaldehyde in biological samples. It utilizes our proprietary fluorogenic sensor AldeLight™ Green, which yields a green fluorescent product upon reacting with formaldehydes. The fluorescence intensity of the signal is directly proportional to the concentration of formaldehyde in solution. This assay can detect as little as 1 µM of formaldehyde using a fluorescence microplate reader at Ex/Em = 410/525 nm. It is convenient for 96-well or 384-well microtiter plate format, and since this assay is continuous and requires no separation step, it can be readily adapted for automation.

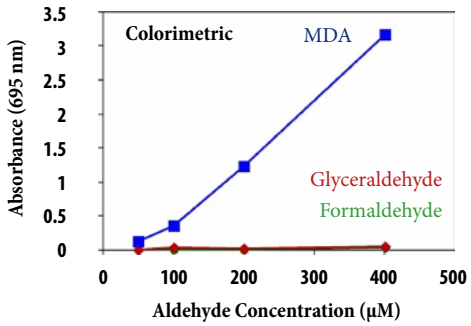

Figure 8.4 MDA dose response was measured with Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit (Cat#10070) on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices).

Malondialdehyde Assays

Malondialdehyde (MDA) is a natural byproduct of lipid peroxidation and is widely used as a key marker for determining oxidative stress and free radical formation. Measurement of MDA has historically relied on reacting it with Thiobarbituric Acid (TBA) to produce MDA-TBA adducts. The resulting adduct can be quantified colorimetrically at 532 nm or fluorimetrically at Ex/Em = 530/550 nm. However, this assay is not specific to MDA and is requires testing under acidic conditions at 90-100°C.

Amplite™ Colorimetric Malondialdehyde Quantitation assay offers a rapid and convenient method for the determination of MDA without the TBARS heating steps. It utilizes our proprietary MDA sensor MDA Blue™, which exhibits little interference from other aldehydes. Upon association with MDA, the sensor generates a blue chromogenic product which is measured using an absorbance microplate reader at 695 nm. Our Amplite™ Fluorimetric Malondialdehyde Quantitation assay utilizes a similar approach for measuring MDA. It uses our proprietary fluorogenic sensor Monoaldelite™ Blue, which upon reacting with MDA generates a strong fluorescence signal. This assay has the capacity to detect as little as 4 µM of MDA using a fluorescence microplate reader at Ex/Em = 365/435 nm.

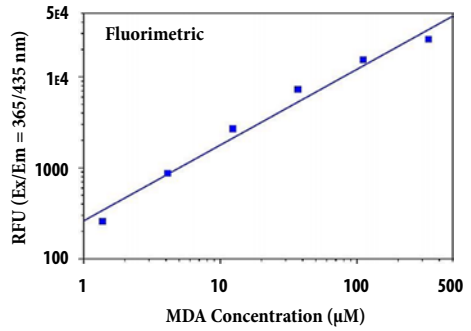

Figure 8.5 MDA dose response was measured with Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit (Cat#10071) on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em=365/435 nm, cutoff=420 nm. As low as 4 µM MDA was detected with 30 minutes incubation (n=3).

Cell Meter™ Intracellular Colorimetric Lipid Peroxidation Assay Kit

Lipid peroxidation is characterized by the oxidative degradation of unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol. Malondialdehyde (MDA), which is one of the most commonly used biomarkers for determining lipid peroxidation, is measured by reacting it with Thiobarbituric Acid (TBA). This reaction yields a detectable product that can be quantified colorimetrically and fluorimetrically. However, this technique is not specific to MDA and requires harsh experimental conditions. Our Cell Meter™ Intracellular Colorimetric Lipid Peroxidation Quantitation assay offers a rapid and convenient method for assessing lipid peroxidation through the quantification of MDA. It utilizes our proprietary MDA sensor MDA Blue™, which exhibits little interference from other aldehydes. Upon association with MDA, the sensor generates a blue chromogenic product which is measured using an absorbance microplate reader at 695 nm.

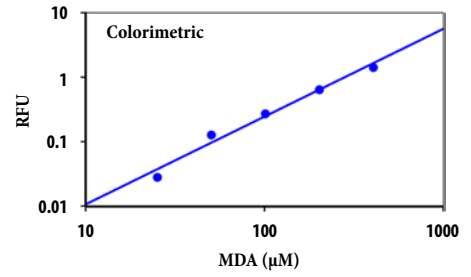

Figure 8.6 MDA dose response was measured with Cell Meter™ Colorimetric Lipid Peroxidation (MDA) Quantitation Kit (Cat#15991) on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices).

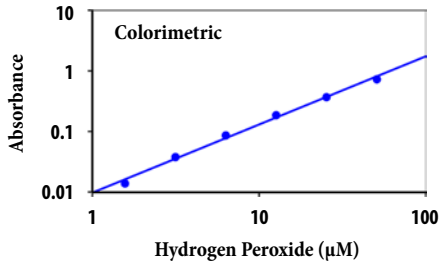

Figure 8.7 H_2O_2 dose response was measured in a white wall/clear bottom 96-well plate with Amplite™ Colorimetric Hydrogen Peroxide Assay Kit (Cat#11500) using a Spetramax absorbance microplate reader (Molecular Devices). As low as 1.5 µM H_2O_2 can be detected with 30 minute incubation (n=3).

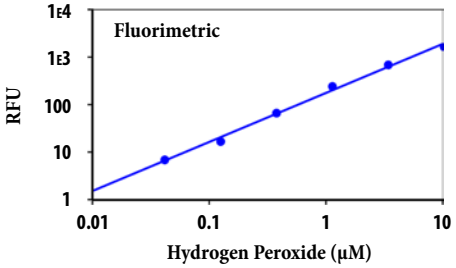

Figure 8.8 H_2O_2 dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat#11502) using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.03 µM H_2O_2 can be detected with 1 minute incubation (n=3).

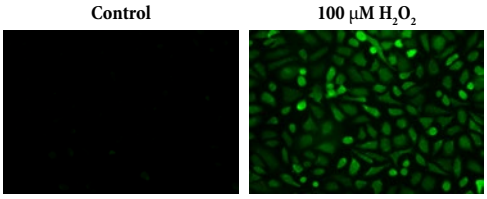

Figure 8.9 Fluorescence images of intracellular hydrogen peroxide in HeLa cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat#11503). HeLa cells were treated without (left) or with (right) 100 µM H_2O_2 at 37 °C for 90 minutes.

Hydrogen Peroxide Assays

In organisms, hydrogen peroxide (H_2O_2) is produced as a toxic by-product of normal aerobic metabolism. It serves as an intracellular signaling messenger and as a key regulator for various oxidative stress-related states. At elevated levels, H_2O_2 is toxic to cells. It can cause oxidative damage to DNA molecules, membrane lipids and proteins, which can lead to mutagenesis and cell death.

Amplite™ Colorimetric Hydrogen Peroxide assay provides a simple, sensitive and HTS-compatible method for the determination of H_2O_2 in solutions and cell extracts. This assay utilizes our proprietary Amplite™ IR peroxidase substrate, which upon H_2O_2 oxidation, yields an intense blue chromogenic product that is pH independent from pH 4 to 10. In this assay, as little as 1.5 µM of H_2O_2 can be detected using an absorbance microplate reader at 650 nm. The near infrared absorption of the Amplite™ IR product enhances assay sensitivity by minimizing background interference from autofluorescing proteins.

Our Amplite™ Fluorimetric Hydrogen Peroxide assay provides an ultrasensitive, one-step fluorimetric method for determining H_2O_2. The Amplite™ IR peroxidase substrate utilized in this assay generates a strong fluorescence upon H_2O_2 oxidation that is pH-independent from pH 4 to 10. This assay can detect as little as 0.03 µM of H_2O_2 using a fluorescence microplate reader at Ex/Em = 640/680 nm. Since this assay is continuous and requires no separation step, it can be readily adapted for automation.

Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit

Our Cell Meter™ Fluorimetric Hydrogen Peroxide assays offer a convenient method for the determination of H_2O_2 in live cells. These assays utilize either of our cell-permeable peroxide sensors, OxiVision™ Blue and OxiVision™ Green to quantify H_2O_2. Upon reacting with H_2O_2, both peroxide sensors generate intense fluorescence that can be detected using a flow cytometer or a fluorescence microscope. OxiVision™ Blue generates a blue fluorescence signal that can be read at Ex/Em = 405/450 nm and observed with a DAPI filter set. OxiVision™ Green generates a green fluorescence signal that can be read at Ex/Em = 490/520 nm and observed with a FITC filter set.

Product Ordering Information For Quantifying Aldehydes, Formaldehydes, MDA and H_2O_2

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
10051	Amplite™ Colorimetric Aldehyde Quantitation Kit	200 Tests	550	N/A
10053	Amplite™ Colorimetric Aldehyde Quantitation Kit *Blue Color*	200 Tests	620	N/A
11500	Amplite™ Colorimetric Hydrogen Peroxide Assay Kit	500 Tests	650	N/A
10070	Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit	200 Tests	695	N/A
10052	Amplite™ Fluorimetric Aldehyde Quantitation Kit	200 Tests	360	450
10057	Amplite™ Fluorimetric Formaldehyde Quantitation Kit *Green Fluorescence*	200 Tests	400	510
10071	Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit	200 Tests	365	435
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 Tests	647	670
15991	Cell Meter™ Intracellular Colorimetric Lipid Peroxidation (MDA) Assay Kit	200 Tests	695	N/A
11505	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence Optimized for Flow Cytometry*	100 Tests	405	450
11504	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence*	200 Tests	405	450
11506	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 Tests	490	530
11503	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*	200 Tests	492	515

Alphabetical Index

Catalog Number Index

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Biomol GmbH
Email: info@biomol.de
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Assay Matrix Pty Ltd.
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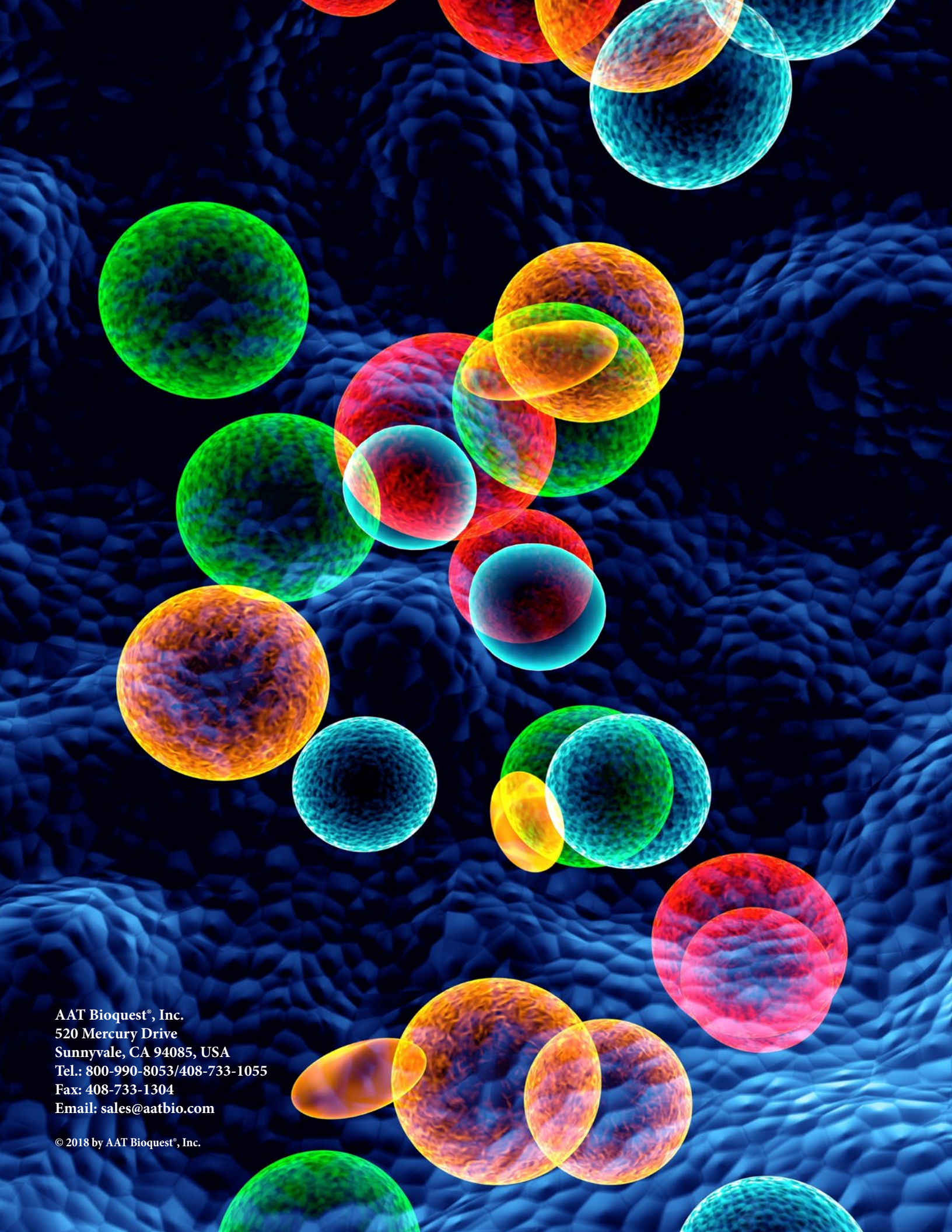

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