Reactive Oxygen Species (ROS) Detection

H_2O_2 · $O_2^{\bullet-}$ · •OH · ClO^- · NO• · $NO_3^{\bullet-}$ · MDA · RSH

biomol
SIE HABEN DIE VISION,
WIR HABEN DIE SUBSTANZ.

AAT Bioquest®
Advancing Assay & Test Technologies

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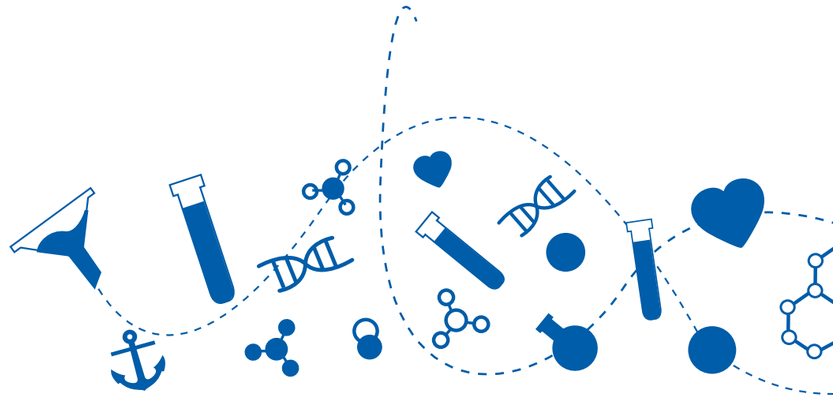

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Custom Products and Services

Our Technologies

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

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

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

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

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

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

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

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

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

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

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

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

Our Services

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

Custom Assay Design and Development

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

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

Custom Conjugation

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

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

Custom Screening

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

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

Custom Synthesis of Fluorophores and Luminophores

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

The Overview of ROS Reagents and Assay Kits

Reactive oxygen species (ROS) are chemically reactive species containing oxygen. Oxygen atom has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, peroxynitrite anion and nitric oxide.

ROS are produced during a number of physiological and pathological processes. Recent evidence has shown these species play a key role as a messenger in normal cell signal transduction and cell cycling even at low levels. For example, as the essential component in cellular defense, ROS are generated in immune cells in response to microbial invasion. At higher levels, these species may damage cellular macromolecules (such as DNA and RNA) and participate in cell apoptosis by activating cell signaling cascades. Many diseases are caused by excessive ROS as a result of an imbalance between radical-generating and radical-scavenging systems, a condition called oxidative stress.

AAT Bioquest offers the most complete product line for detecting ROS. Our ROS products include novel fluorescent indicators and assay kits to detect various reactive oxygen species and related biochemical molecules with high sensitivity and selectivity. Our fluorescent ROS probes enable both the total detection of intracellular ROS (Table 1), and the selective measurement of individual ROS in live cells with high selectivity (Table 2). They are optimized for multiplexing applications by selecting desired excitation and emission wavelengths for flow cytometric analysis and fluorescence imaging of live cells from a broad spectrum of our ROS detection products.

Table 1. Intracellular ROS Detection Reagents and Assay Kits

Reactive Oxygen Species (ROS)		ROS Brite™ 570	ROS Brite™ 670	ROS Brite™ 700	ROS Brite™ HDCF	Amplite™ ROS Green	Amplite™ ROS Red
Hydrogen peroxide	H_2O_2	+	+	+	+++	+++	+++
Hydroxyl radical	•OH	++	++	++	+	+	+
Tert-butyl-Hydroperoxide	TBHP	+	+	+	+	+	+
Hypochlorous acid	HOCl	-	+	++	-	+	-
Superoxide anion	$O_2^{\cdot-}$	+	++	++	-	-	-
Nitric oxide	NO	-	-	-	-	-	-
Peroxynitrite anion	$ONOO^-$	-	-	-	-	-	-
Cat#		22902	22903	16004	16053	22900, 22904	22901

Table 2. Selective ROS Detection Reagents and Assay Kits

Reactive Oxygen Species (ROS)		Nitrixyte™ Orange	Nitrixyte™ Red	Nitrixyte™ NIR	DAX-J2™ PON Green	OxiVision™ Green	OxiVision™ Blue	MitoROS™ 520	MitoROS™ 580	MitoROS™ OH580
Hydrogen peroxide	H_2O_2	-	-	-	-	+++	+++	-	-	-
Hydroxyl radical	•OH	-	-	-	-	-	-	-	-	+++
Tert-butyl-Hydroperoxide	TBHP	-	-	-	-	-	-	-	-	-
Hypochlorous acid	HOCl	-	-	-	-	-	-	-	-	-
Superoxide anion	$O_2^{\cdot-}$	-	-	-	-	-	-	+++	+++	-
Nitric oxide	NO	+++	+++	+++	-	-	-	-	-	-
Peroxynitrite anion	$ONOO^-$	-	-	-	+++	-	-	-	-	-
Cat#		16350 16351	16356	16359 16360	16315 16317	11503 11506	11504 11505	16060	22970 22971	16055

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Hydrogen Peroxide Detection

Hydrogen Peroxide Detection by ADHP and Its Analogs

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

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

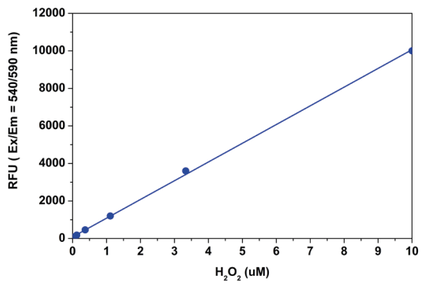

Figure 1. H_2O_2 dose responses were measured in a 384-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501). As low as 0.03 µM H_2O_2 was detected with 30 minutes incubation (n=3).

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502) uses our unique Amplite™ IR peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. Amplite™ IR generates the fluorescence that is pH-independent from pH 4 to 10. It is a superior alternative to ADHP (Amplex® Red) for the detections that require low pH where ADHP has reduced fluorescence. In addition, Amplite™ IR generates a product that has maximum absorption at 647 nm with maximum emission at 670 nm. The near infrared fluorescence minimizes the assay background that is often caused by the autofluorescence of biological samples. The kit can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502) provides a sensitive, one-step fluorimetric assay to detect as little as 3 picomoles of H_2O_2 in a 100 µL assay volume (30 nM).

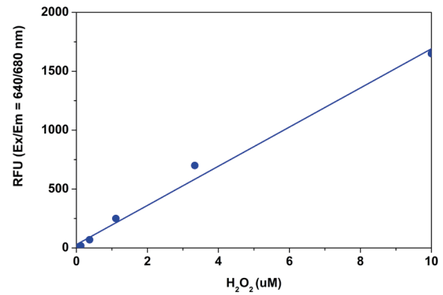

Figure 2. H_2O_2 dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502). As low as 0.03 µM H_2O_2 was detected.

Table 3. Hydrogen Peroxide Assay Kits

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

Intracellular Hydrogen Peroxide Detection Kits

Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503) uses our unique Amplite™ Green to quantify hydrogen peroxide in live cells. Cell-permeable Amplite™ Green generates green fluorescence when it reacts with hydrogen peroxide. The kit is in an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit provides a sensitive, one-step fluorimetric assay to detect H_2O_2 in live cells. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 490/520 nm for H_2O_2 detection or a fluorescence microscope.

Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kits (Cat# 11504, 11505 & 11506) use our unique cell-permeable OxiVision™ peroxide sensors to quantify hydrogen peroxide in living cells. The kits provide a sensitive tool to monitor hydrogen peroxide level and they are optimized to be used in flow cytometry.

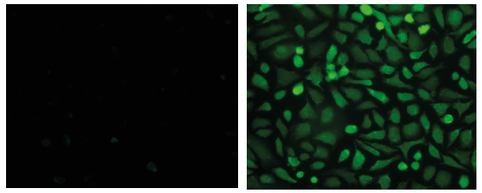

Figure 3. Images of live CHO-K1 cells in a 96-well plate. Live CHO-K1 cells were stained with Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503). Left: Control cells. Right: Cells treated with 100 µM H_2O_2 at room temperature for 5 minutes.

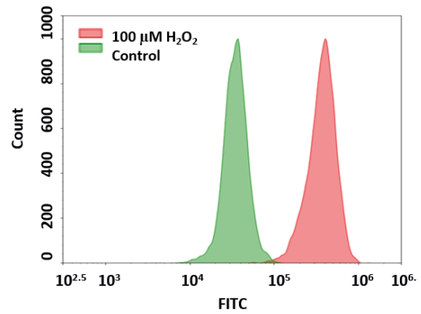

Figure 4. Detection of hydrogen peroxide in Jurkat cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11506). Jurkat cells were stained with OxiVision™ Green peroxide sensor for 30 minutes and treated with 100 µM hydrogen peroxide at 37 °C for 90 minutes. Cells stained with OxiVision™ Green peroxide sensor but without hydrogen peroxide treatment were used as control.

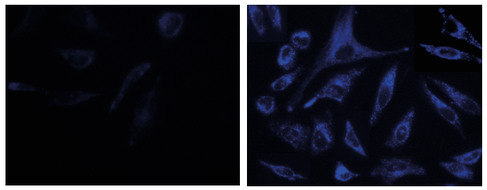

Figure 5. Fluorescence images of intracellular hydrogen peroxide in HeLa cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11504). HeLa cells at 10,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. Control (left): Cells were stained with OxiVision™ Blue peroxide sensor but without hydrogen peroxide treatment. 100 µM H_2O_2 (right): HeLa cells were stained with OxiVision™ Blue peroxide sensor for 30 minutes and treated with 100 µM hydrogen peroxide at 37 °C for 90 minutes. The fluorescence signals were measured using fluorescence microscope with a DAPI filter.

Intracellular Hydrogen Peroxide Detection Probes

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

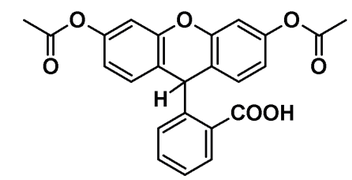

Figure 6. The chemical structure of dihydrofluorescein diacetate (Cat# 15203).

2',7'-Dichlorodihydrofluorescein diacetate (also called 2',7'-dichlorofluorescein diacetate, Cat# 15204) works similarly to dihydrofluorescein diacetate (Cat# 15203). 2',7'-Dichlorodihydrofluorescein diacetate has lower pK_a, making this probe superior for the assays that require low pH.

Dihydrorhodamine 123 (DHR 123, Cat# 15206) is by far the most-used probe for the measurement of intracellular H_2O_2. DHR 123 is oxidized directly to rhodamine 123, which is excitable at 488 nm and emits at 515 nm in the same emission range as FITC. It is widely used in human neutrophils, human eosinophils, HL60 cells, rat mast cells, guinea pig neutrophils, cultured chondrocytes, rat brain, rat renal proximal tubular cells, mesangial cells and L929 cells. In combination with other fluorescent reagents (such as surface receptor analysis using fluorescent antibodies, cell viability using propidium iodide, and calcium indicators), this probe can be used for multiplex measurements.

Table 4. Intracellular Hydrogen Peroxide Detection Assay Kits and Probes

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
11503	Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*	200 tests	492	515
11504	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence*	100 tests	405	450
11505	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence Optimized for Flow Cytometry*	100 tests	405	450
11506	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	490	530
15204	2',7'-Dichlorodihydrofluorescein Diacetate [2',7'-Dichlorofluorescein Diacetate]	25 mg	504	529
15203	Dihydrofluorescein Diacetate [Fluorescein Diacetate]	25 mg	490	514
15206	Dihydrorhodamine 123	10 mg	507	529
15207	Dihydrorhodamine 123	5x1 mg	507	529

Catalase Detection

Catalase is a common antioxidant heme-containing redox enzyme found in nearly all living organisms that are exposed to oxygen. The enzyme is concentrated in the peroxisome subcellular organelles. Hydrogen peroxide is an ROS that is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. By preventing the excessive buildup of H_2O_2, catalase allows important cellular processes which produce H_2O_2 as a by-product to take place safely.

Amplite™ Fluorimetric Catalase Assay Kit (Cat# 11306) provides a quick and sensitive method for the measurement of catalase activity. Catalase reacts with H_2O_2 to produce water and oxygen (O_2). Amplite™ Red used in the assay kit reacts with H_2O_2 to generate a red fluorescent product. Therefore the reduction in fluorescence intensity is proportional to catalase activity. Amplite™ Red enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Catalase Assay Kit, as low as 30 mU/mL catalase was detected in a 100 µL reaction volume.

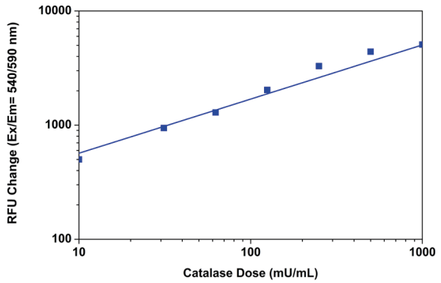

Figure 7. Catalase dose responses were measured with Amplite™ Fluorimetric Catalase Assay Kit (Cat# 11306) in a 96-well solid black plate. As low as 30 mU/mL catalase was detected with 30 minutes incubation (n=3).

Table 5. Catalase Detection Assay Kit

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
11306	Amplite™ Fluorimetric Catalase Assay Kit	200 tests	571	585

Peroxidase Detection

Peroxidase is a small molecule (MW ~40 kDa) that can usually be conjugated to an antibody. Due to its small size, it rarely causes steric hindrance problems with antibody/antigen complex formation. Peroxidase is inexpensive compared to other labeling enzymes. The major disadvantage associated with peroxidase is its low tolerance to many preservatives, such as sodium azide, that inactivates peroxidase activity even at low concentration. HRP conjugates are extensively used as secondary detection reagents in ELISAs, immuno-histochemical techniques as well as Northern, Southern and Western blot analyses.

Amplite™ Colorimetric Peroxidase Assay Kit (Cat# 11551) uses Amplite™ Blue, our ultrasensitive chromogenic HRP substrate. Amplite™ Blue is a chromogenic peroxidase substrate that is much more sensitive to both H_2O_2 and peroxidase than other chromogenic peroxidase substrates such as TMB, ABTS, OPD and K-Blue®. Amplite™ Blue generates a highly absorptive material that has maximum absorption at 664 nm. This near infrared absorption minimizes the background absorption often caused by the autoabsorption of biological samples that rarely absorb light beyond 600 nm. The signal can be easily read by an absorbance microplate reader at 664±5 nm.

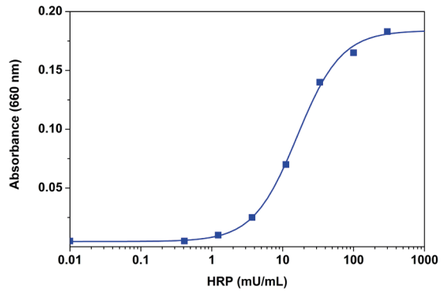

Figure 8. HRP dose responses were measured with Amplite Colorimetric Peroxidase Assay Kit (Cat# 11551) in a 96-well white wall/clear bottom plate. As low as 3 mU/mL peroxidase was detected.

Amplite™ Fluorimetric Peroxidase Assay Kits (Cat# 11552 & 11553) are quick (10 min) HRP assays in a one-step, homogeneous, no wash assay system. They can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screening of oxidase inhibitors. The kits provide an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11552) uses fluorogenic Amplite™ Red HRP substrate to quantify peroxidase in solutions. It can be used for ELISAs, characterizing kinetics of enzyme reaction, and high throughput screenings. The kit provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. As low as 10 µU/mL HRP was detected. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553) uses Amplite™ IR, our near infrared fluorogenic HRP substrate. Amplite™ IR generates a substance that has maximum absorption of 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background often caused by the autoabsorption and/or autofluorescence of biological samples that rarely absorb light beyond 600 nm. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 600 to 650/650 to 690 nm (maximum Ex/Em = 640/680 nm) or an absorbance micropalte reader at 647 ± 5 nm. As low as 1 mU/mL HRP was detected with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553).

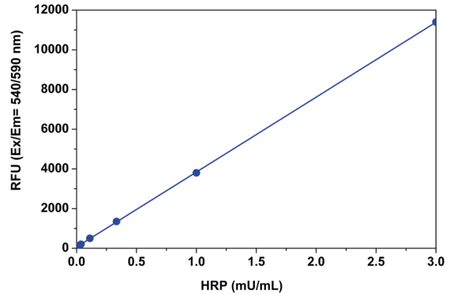

Figure 9. HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11552) in a 384-well black plate. As low as 10 µU/mL peroxidase was detected with 30 minutes incubation (n=3).

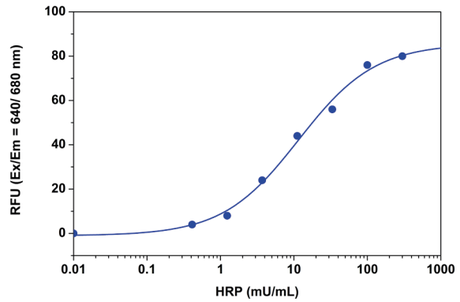

Figure 10. HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553) in a solid black 384-well plate using a Gemini fluorescence microplate reader. As low as 1 mU/mL peroxidase was detected with 30 minutes incubation (n=3).

Myeloperoxidase (MPO), most abundantly present in neutrophils and monocytes, is a green hemoprotein having peroxidase activity. It catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates and plays an important role in the oxygen-dependent killing of tumor cells and microorganisms. MPO deficiency is a hereditary deficiency of the enzyme, which predisposes a person to immune deficiency. There is considerable interest in the development of therapeutic MPO inhibitors.

Amplite™ Myeloperoxidase Assay Kit (Cat# 11301) provides a quick and sensitive method for the measurement of myeloperoxidase in solution and in cell lysates. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Myeloperoxidase Assay Kit, as low as 0.1 mU/mL myeloperoxidase was detected in a 100 µL reaction volume.

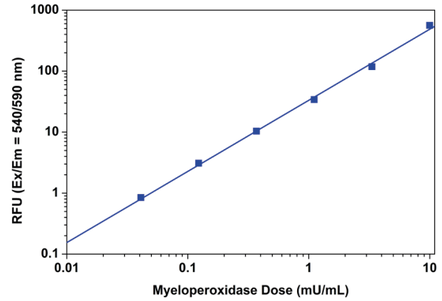

Figure 11. Myeloperoxidase dose responses were measured with Amplite™ Fluorimetric Myeloperoxidase Assay Kit (Cat# 11301) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.1 mU/mL myeloperoxidase was detected with 60 minutes incubation (n=3).

Amplite™ ADHP (Cat# 11000) has the same chemical structure as Amplex® Red. It is a sensitive fluorogenic peroxidase substrate that has much lower background than the materials from other commercial vendors. ADHP generates highly fluorescent resorufin that has maximum absorption at 571 nm and maximum emission at 585 nm. Unlike other HRP substrates, such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of ADHP is minimal. So far ADHP has been known as the most sensitive and stable fluorogenic probe for detecting HRP and H_2O_2. ADHP has been widely used to detect HRP in many immunoassays. On the other hand, ADHP can also be used to detect trace amount of H_2O_2. The ADHP-based H_2O_2 detection is at least one order of magnitude more sensitive than the commonly used scopoletin assay for H_2O_2. Because H_2O_2 is produced in many enzymatic redox reactions, ADHP can be used in coupled enzymatic reactions to detect the activity of many oxidases and/or related enzymes/substrates or cofactors, such as glucose, acetylcholine and cholesterol, L-glutamate, amino acids.

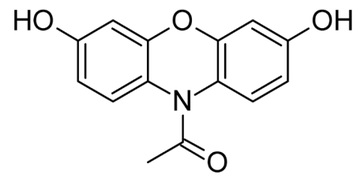

Figure 12. The chemical structure of Amplite™ ADHP (Cat# 11000).

Horseradish peroxidase (HRP) and HRP conjugates facilitate ABTS oxidation in the presence of hydrogen peroxide, turning ABTS into its blue-green oxidized product. This chromogenic reaction is widely used to quantify HRP activity in ELISA assays. The oxidized ABTS product has absorption maximum at 420 nm that can easily be followed with a spectrophotometer. ReadiUse™ ABTS Substrate Solution (Cat# 11001) is optimized for ELISA assays that use HRP or HRP-labeled conjugates and hydrogen peroxide in microwell plates or test tubes. ABTS solution allows HRP reactions to be completed in a single addition. The assay solution changes the color to light green upon its reaction with HRP or HRP conjugates in the presence of hydrogen peroxide.

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ReadiUse™ TMB Substrate Solution (Cat# 11003) is a premixed solution of TMB substrate with hydrogen peroxide. It produces a blue product upon interaction with HRP or HRP conjugates without the addition of hydrogen peroxide. The soluble blue product can be quantitated at 650 nm. Use of a stop solution enhances sensitivity 2-4 fold and the resulting yellow solution can be read at 450 nm. ReadiUse™ TMB Substrate Solution provides a convenient and ultra-sensitive quantitative substrate system.

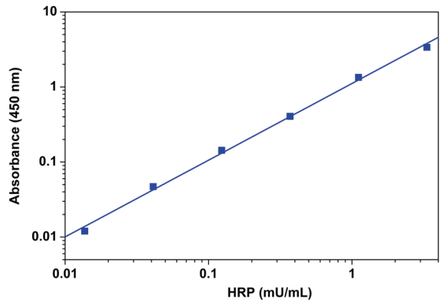

Figure 13. HRP dose responses were measured with ReadiUse™ TMB Substrate Solution (Cat# 11003) in a clear 96-well plate. As low as 3 µU/well peroxidase was detected with 10 minutes incubation.

Table 6. Peroxidase Detection Assay Kits and Probes

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
11000	Amplite™ ADHP	25 mg	571	585
11551	Amplite™ Colorimetric Peroxidase Assay Kit *Blue Color*	500 tests	664	N/A
11552	Amplite™ Fluorimetric Peroxidase Assay Kit *Red Fluorescence*	500 tests	571	585
11553	Amplite™ Fluorimetric Peroxidase Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11301	Amplite™ Fluorimetric Myeloperoxidase Assay Kit *Red Fluorescence*	200 tests	571	585
11001	ReadiUse™ ABTS Substrate Solution	1 L	420	N/A
11003	ReadiUse™ TMB Substrate Solution	1 L	650	N/A
11010	Signal Guard™ HRP Conjugate Stabilizer	50 mL	N/A	N/A

MDA Quantitation

Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are natural byproducts of lipid peroxidation. Lipid peroxidation or reaction of oxygen with unsaturated lipids can be described as a process under which oxidants such as free radicals take electron from the lipids (generally in cell membranes), resulting in cell damage. As the most popular and reliable biomarker for lipid peroxidation, MDA has been widely used for many years to determine oxidative stress in clinical situations. Therefore, quantification of MDA is essential to assess oxidative stress in pathophysiological processes.

The Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit (Cat#10070) offers a quick and continent method to measure MDA without heating steps. The Monoaldite™ Blue reacts with MDA to generate a blue color. The assay can be measured by an absorbance microplate reader at 650 nm.

Similarly our Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit (Cat#10071) provides a quick and sensitive method to measure MDA without heating steps. Monoaldelite™ Blue itself is nearly non-fluorescent, but generates strong blue fluorescence upon reacting with MDA.

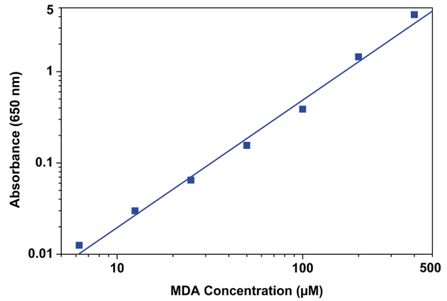

Figure 14. MDA dose response was measured with Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit (Cat# 10070) on a 96-well clear bottom microplate using a SpectraMax® microplate reader (Molecular Devices). As low as 12.5 µM MDA was detected with 30 minutes incubation (n=3). (Note: The absorbance background increases with time, thus it is important to subtract the absorbance of the blank wells for each data point.)

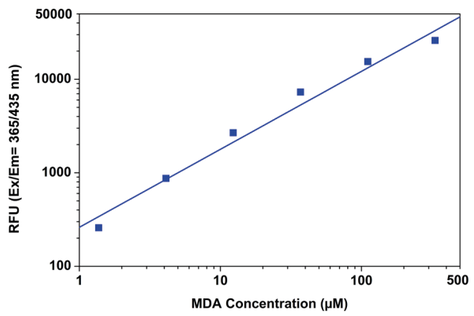

Figure 15. MDA dose response was measured with Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit (Cat# 10071) on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices). As low as 3 µM MDA was detected with 30 minutes incubation (n=3). (Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.)

Table 7. Malondialdehyde (MDA) Quantitation Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
10072	Amplite™ Colorimetric Malondialdehyde (MDA) ELISA Kit	100 tests	550	N/A
10070	Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit	200 tests	550	N/A
10073	Amplite™ Fluorimetric Malondialdehyde (MDA) ELISA Kit	100 tests	571	585
10071	Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit	200 tests	360	450

Table 8. Hydroxyl Radical Assay Kit

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
16055	Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit *Red Fluorescence*	200 tests	576	598

Hydroxyl Radical Detection

The detection of intracellular hydroxyl radical (•OH) is of central importance to the understanding of proper cellular redox regulation and the impact of its dysregulation on various pathologies. The hydroxyl radical is one of the reactive oxygen species (ROS) that are highly reactive with other molecules to achieve stability. In general, the hydroxyl radical is considered to be a harmful byproduct of oxidative metabolism, which can cause molecular damage in living systems. It shows an average lifetime of 10^{-9} ns and can react with nearly every biomolecule, such as nuclear DNA, mitochondrial DNA, proteins and membrane lipids.

Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit (Cat# 16055) is optimized for detecting hydroxyl radical in mitochondria. MitoROS™ OH580 used in the kit is a live-cell permeant probe that can rapidly and selectively target hydroxyl radical in live cells. It generates red fluorescence when it reacts with •OH, and can be easily read at Ex/Em= 540/590 nm. Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit provides a sensitive fluorimetric probe to detect •OH in live cells with one hour incubation. This kit can be used for fluorescence microplate readers and fluorescence microscopy applications.

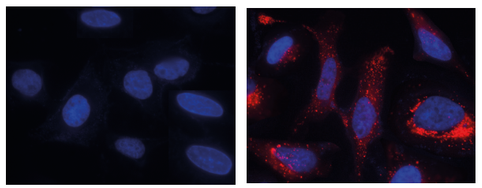

Figure 16. Fluorescence images of hydroxyl radical measurements in HeLa cells using Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit (Cat# 16055). Control (left): HeLa cells were kept in 1X HBSS buffer without treatment. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530). Fenton Reaction (right): Cells were treated with 10 µM $CuCl_2$ and 100 µM H_2O_2 in 1X HBSS buffer at 37 °C for 1 hour.

Superoxide Detection

Hydroethidine (Cat# 15200), a redox-sensitive probe, has been widely used to detect intracellular superoxide anion ($O_2^{\cdot-}$). It is a common assumption that the reaction between superoxide and hydroethidine results in the formation of a two-electron oxidized product, ethidium, which binds to DNA and leads to the enhancement of fluorescence (excitation, 500 - 530 nm; emission, 590 - 620 nm). However, the mechanism of hydroethidine oxidation by the superoxide anion still remains unclear. Hydroethidine operates effectively as a probe for the measurement of reactive oxygen species. The dye enters cells freely and is oxidized to ethidium bromide. The probe has been used extensively with NK cells and as a vital dye for identification of proliferation and hypoxic cells in tumors. Studies have been performed using neutrophils and endothelial cells as well as HL60 cells and macrophages. A major advantage of this probe is its ability to distinguish between superoxide and H_2O_2.

MitoROS™ 580 (Cat# 16052) is a superoxide-sensitive dye that is localized in mitochondria upon loading into live cells. Oxidation of MitoROS™ 580 by superoxide generates red fluorescence. MitoROS™ 580 can be used for monitoring superoxide in mitochondria either with a fluorescence microscope or a fluorescence flow cytometer. MitoROS™ 580 reagent permeates live cells where it selectively targets mitochondria. It is rapidly oxidized by superoxide. It is less likely to be oxidized by other reactive oxygen species (ROS) and reactive nitrogen species (RNS). The oxidized product is highly fluorescent in cells. MitoROS™ 580 provides a valuable tool for investigating oxidative stress in various pathologies.

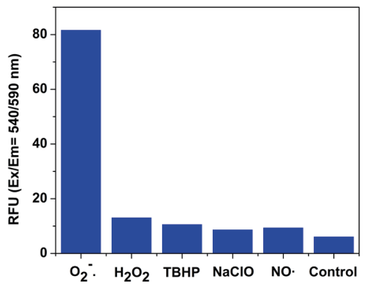

Figure 17. Fluorescence response of MitoROS™ 580 (10 µM, Cat# 16052) to different reactive oxygen species (ROS) and reactive nitrogen species (RNS). The fluorescence intensities were monitored at Ex/Em = 540/590 nm.

The detection of intracellular mitochondrial superoxide is of great importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies. Cell Meter™ Fluorimetric Mitochondrial Superoxide Detection Kits (Cat# 16060, 22970 & 22971) use our unique MitoROS™ superoxide indicators, to quantify superoxide level in live cells. MitoROS™ sensors are cell permeant and can rapidly and selectively detect superoxide in mitochondria. The Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kits provide a sensitive, one-step fluorimetric assay to detect mitochondrial superoxide in live cells with one hour incubation.

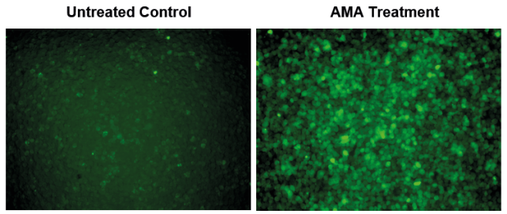

Figure 18. Fluorescence images of superoxide measurement in macrophage cells using Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit (Cat#16060). RAW 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment: Cells were treated with 5 µM Antimycin A (AMA) at 37 °C for 2 hours, then incubated with MitoROS™ 520 for 1 hour. Untreated Control: RAW 264.7 cells were incubated with MitoROS™ 520 at 37 °C for 1 hour without AMA treatment.

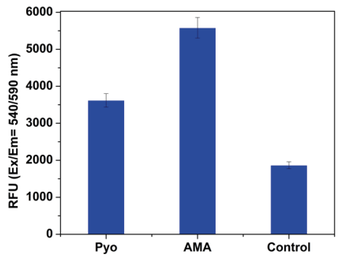

Figure 19. Detection of intracellular superoxide in HeLa cells using Cell Meter™ Fluorimetric Mitochondrial Superoxide Detection Kit (Cat#22971). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were incubated with 50 µM Pyocyanin (Pyo); 50 µM Antimycin A (AMA) or without treatment (Control) at 37 °C for 30 minutes. Cells were then incubated with MitoROS™ 580 (Cat# 16052) at 37 °C for 1 hour. The fluorescence signal was monitored at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode.

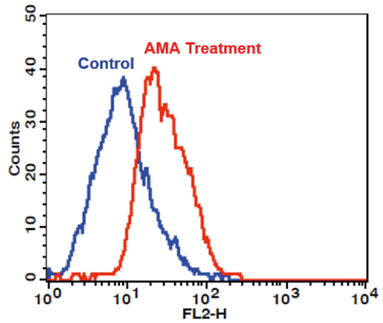

Figure 20. Detection of intracellular superoxide in Jurkat cells using Cell Meter™ Fluorimetric Mitochondrial Superoxide Detection Kit (Cat# 22970). AMA Treatment (Red): Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Control (Blue): Cells were incubated with MitoROS™ 580 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).

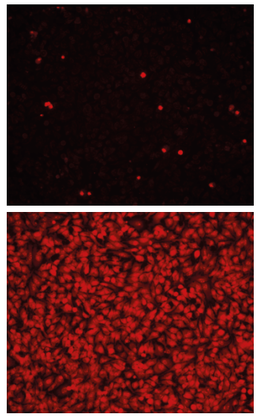

Figure 21. Fluorescence images of superoxide measurement in HeLa cells using Cell Meter™ Fluorimetric Mitochondrial Superoxide Detection Kit (Cat#22971). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment (bottom): Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 (Cat# 16052) for 1 hour. Untreated Control (top): HeLa cells were incubated with MitoROS™ 580 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was measured with a TRIC filter.

Table 9. Intracellular Superoxide Detection Assay Kits and Probes

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
16060	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit *Green Fluorescence*	200 tests	509	534
22970	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit *Optimized for Flow Cytometry*	100 tests	540	590
22971	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit *Optimized for Microplate Reader*	200 tests	540	590
15200	Hydroethidine	25 mg	518	605
16052	MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochnodria*	500 tests	510	580

SOD Detection

Superoxide dismutases (SODs) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide is one of the main reactive oxygen species in cells. It is a substantial contributor to pathology associated with neurodegenerative diseases, ischemia reperfusion injury, atherosclerosis and aging. SODs are an important antioxidant defense in nearly all cells exposed to superoxide radicals. In fact, mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation.

Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit (Cat# 11305) provides a quick and sensitive method for the measurement of SOD activity in solutions. In the assay, xanthine is converted to superoxide radical ions, uric acid and hydrogen peroxide by xanthine oxidase (XO). Superoxide reacts with SOD Orange™ to generate a product that absorbs at around 560 nm. SOD inhibits the reaction of SOD Orange™ with superoxide, thus reduces the absorption at 560 nm. The reduction in the abosoption of SOD Orange™ at 560 nm is proportional to SOD activity. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format.

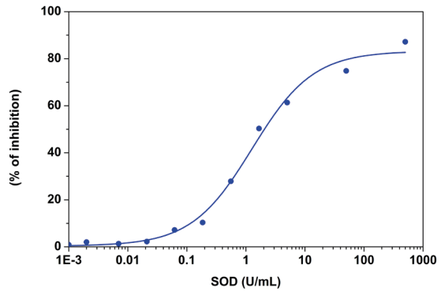

Figure 22. SOD dose responses were measured with Amplite™ Colorimetric Superoxide Dismutase Assay Kit (Cat# 11305). As low as 0.1 U/mL SOD was detected with 60 minutes incubation (n=3).

Table 10. Superoxide Dismutase Detection Assay Kit

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
11305	Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit	200 tests	560	N/A

Xanthine Assays

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

Amplite™ Xanthine Assay Kits (Cat# 13842 & 13843) provide a quick and ultrasensitive method for the measurement of xanthine. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Xanthine is oxidized to uric acid in the presence of xanthine oxidase to release hydrogen peroxide, which can be specifically measured with Amplite™ Red by an absorbance microplate reader at 576±5 nm or by a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With Amplite™ Colorimetric Xanthine Assay Kit (Cat# 13842), as low as 1.2 µM xanthine was detected in a 100 µL reaction volume. With Amplite™ Fluorimetric Xanthine Assay Kit (Cat# 13843), as low as 0.14 µM xanthine was detected in a 100 µL reaction volume.

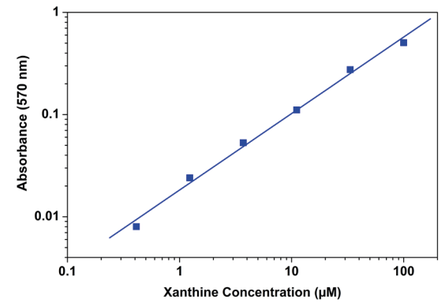

Figure 23. Xanthine dose responses were measured with Amplite™ Colorimetric Xanthine Assay Kit (Cat# 13842) in a 96-well clear bottom plate. As low as 1.2 µM xanthine was detected with 30 minutes incubation (n=3).

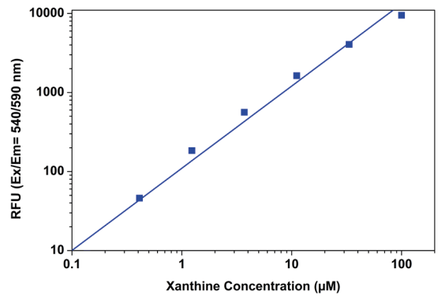

Figure 24. Xanthine dose response was measured with Amplite™ Fluorimetric Xanthine Assay Kit (Cat# 13843) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.14 µM xanthine was detected with 30 minutes incubation time (n=3).

Xanthine oxidase (XO) is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. It plays an important role in the catabolism of purines. Xanthine oxidase is normally found in liver and jejunum. During severe liver damage, xanthine oxidase is released into blood, so a blood assay for XO is a way to determine if liver damage has happened. Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in blood and can cause health problems, such as renal failure.

Amplite™ Xanthine Oxidase Assay Kits (Cat# 11304 & 11307) provide a quick and ultrasensitive method for the measurement of xanthine oxidase activities. In the assay, xanthine oxidase catalyzes the oxidation of purine bases, hypoxanthine or xanthine to uric acid and superoxide , which spontaneously degrades to hydrogen peroxide (H_2O_2). The kits use our Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With the Fluorimetric Kit 11304, as low as 14 µU/mL xanthine oxidase was detected in a 100 µL reaction volume. The color signal can be easily read at ~570 nm with an absorbance microplate reader. With the Colorimetric Kit 11307, we have detected as little as 0.12 mU/mL xanthine oxidase in a 100 µL reaction volume.

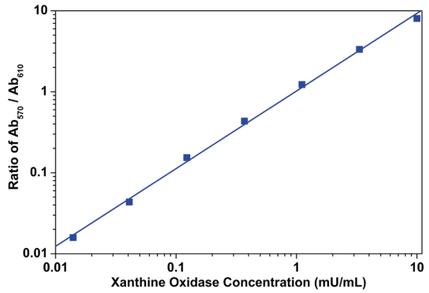

Figure 25. Xanthine oxidase dose response was measured with Amplite™ Colorimetric Xanthine Oxidase Assay Kit (Cat# 11307) in a white or black wall/clear bottom 96-well microplate. As low as 0.12 mU/mL xanthine oxidase was detected.

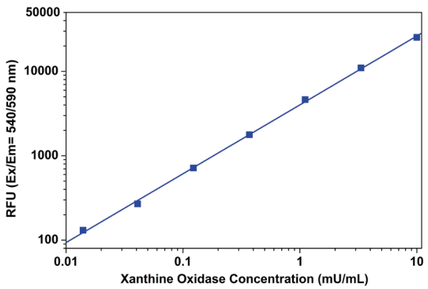

Figure 26. Xanthine oxidase dose response was measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit (Cat# 11304) in a 96-well black solid plate. As low as 14 µU/mL xanthine oxidase was detected.

Table 11. Xanthine Detection Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
13842	Amplite™ Colorimetric Xanthine Assay Kit	200 tests	575	N/A
11307	Amplite™ Colorimetric Xanthine Oxidase Assay Kit *Red Fluorescence*	200 tests	570	N/A
13843	Amplite™ Fluorimetric Xanthine Assay Kit	200 tests	571	585
11304	Amplite™ Fluorimetric Xanthine Oxidase Assay Kit *Red Fluorescence*	200 tests	571	585

Hypochlorite Assays

Hypochlorite anion (ClO-) and its protonated form, hypochlorous acid (HClO) are critical reactive oxygen species (ROS) in biological systems. Uncontrolled production of hypochlorite (hypochlorous acid) can lead to tissue damage and diseases including arthritis, renal failure and cancers. In addition, sodium hypochlorite (NaClO) has been widely used as a bleaching agent for surface cleaning, odor removal and water disinfection in our daily lives. Exposure to large amount of sodium hypochlorite can lead to poisoning with the symptoms of serious breathing problems, stomach irritation, redness and pain on skin and eye.

Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit (Cat# 13846) offers a sensitive fluorescence-based assay for measuring hypochlorite (hypochlorous acid) with high specificity. Upon selective reaction with hypochlorite (hypochlorous acid) the weakly fluorescent Oxirite™ Hypochlorite Sensor generates a strongly fluorescent product that gives more than 100-fold fluorescence enhancement. The fluorescence signal can be measured by a fluorescence microplate reader at Ex/Em= 540/590 nm. With Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit, as low as 3 µM hypochlorite was detected in a 100 µL reaction volume.

Amplite™ Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit (Cat# 13845) uses Oxirite™ Hypochloride Sensor, which selectively reacts with hypochlorite (hypochlorous acid) to generate a red color product. The assay can be measured with an absorbance microplate reader at around 550 nm.

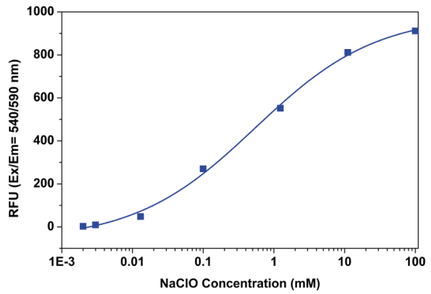

Figure 27. Hypochlorite was measured with Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit (Cat# 13846). As low as 0.003 mM (~3 µM) sodium hypochlorite (NaClO) was detected with 10-30 minutes incubation (n=3).

Table 12. Hypochlorite Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
13845	Amplite™ Colorimetric Hypochlorite (Hypochlorous Acid) Assay Kit	200 tests	575	N/A
13846	Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit	200 tests	571	585

Total ROS Detection

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

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

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

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

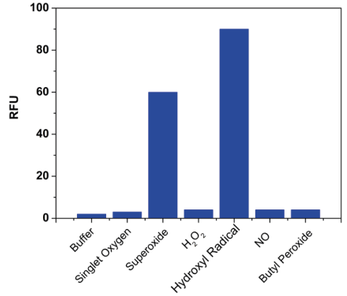

Figure 28. The responses of ROS Brite™ 570 (Cat# 16000) to different ROS species.

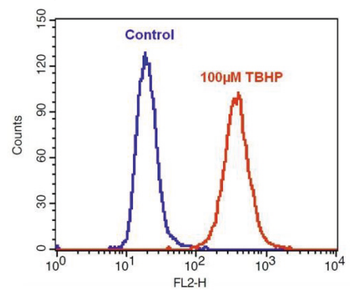

Figure 29. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 570 (Cat# 16000) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

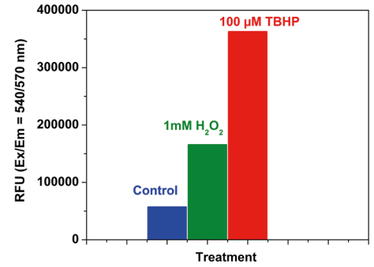

Figure 30. Detection of ROS in HeLa cells. The cells were untreated (control) or treated with 1 mM H_2O_2 or 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. ROS Brite™ 570 (Cat# 16000) (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence signals were monitored at Ex/Em = 540/570 nm (cut off at 550 nm) with bottom read mode.

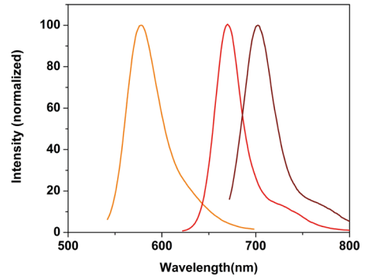

Figure 31. The fluorescence spectra of ROS Brite™ 570 (Yellow, Cat# 16000), ROS Brite™ 670 (Orange, Cat# 16002) and ROS Brite™ 700 (Red, Cat# 16004).

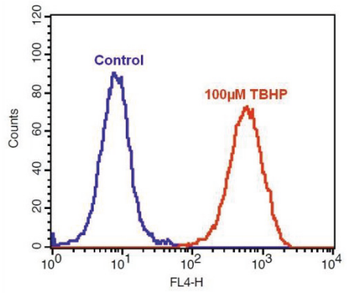

Figure 32. Detection of ROS in Jurkat cells. Jurkat cells were untreated (Blue) or treated with 100 µM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and loaded with ROS Brite™ 670 (Cat# 16002) for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL4 channel.

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

investigating oxidative stress in various pathologies.

ROS Brite™ APF (Cat# 16050) and ROS Brite™ HPF (Cat# 16051) are fluorogenic probes to measure hydroxyly radical in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant ROS Brite™ APF and HPF reagents are nonfluorescent and produce bright green fluorescence upon reaction with hydroxyl radical. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. In the presence of peroxidase, APF also reacts with hydrogen peroxide. APF has good selectivity to hydroxyl radical compared to other ROS. APF and HPF show relatively high resistance to light-induced oxidation. APF will also react with the hypochlorite anion.

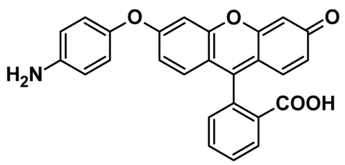

Figure 33. The chemical structure of ROS Brite™ APF (Cat# 16050).

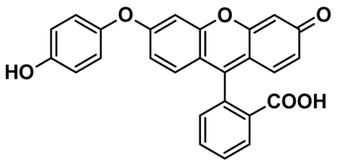

Figure 34. The chemical structure of ROS Brite™ HPF (Cat# 16051).

Table 13. Total ROS Activity Probes

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
16000	ROS Brite™ 570 *Optimized for Detecting Reactive Oxygen Species (ROS)*	1 mg	556	566
16002	ROS Brite™ 670 *Optimized for Detecting Reactive Oxygen Species (ROS)*	1 mg	658	675
16004	ROS Brite™ 700 *Optimized for in Vivo Imaging*	1 mg	680	706
16050	ROS Brite™ APF *Optimized for Detecting Reactive Oxygen Species (ROS)*	1 mg	492	515
16053	ROS Brite™ DHCF	1 mg	560	574
16051	ROS Brite™ HPF *Optimized for Detecting Reactive Oxygen Species (ROS)*	1 mg	492	515

Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kits (Cat# 22900, 22901, 22902 & 22903) provide a sensitive, one-step fluorimetric assay to detect intracellular ROS in live cells. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read using either a fluorescence microplate reader or a fluorescence microscope. Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kits are in an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22904) is optimized for flow cytometry applications, its signal can be detected at Ex/Em = 490/520 nm (FL1 channel).

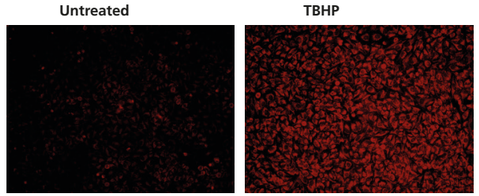

Figure 35. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22902) in a Costar 96-well black wall/clear bottom plate. Left: Untreated control cells. Right: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

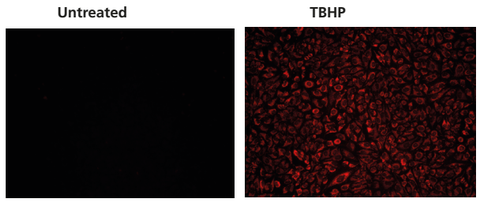

Figure 36. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22903) in a Costar 96-well black wall/clear bottom plate. Left: Untreated control cells. Right: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

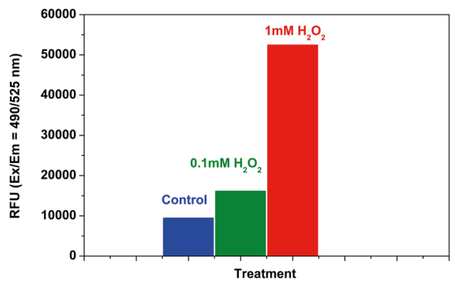

Figure 37. Detection of ROS in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22900). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was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

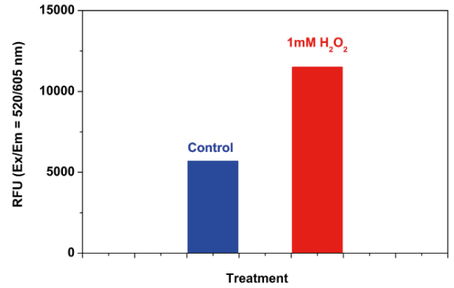

Figure 38. Detection of ROS in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22901). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. And then the cells were treated with or without 1mM H_2O_2 for 2 hours.

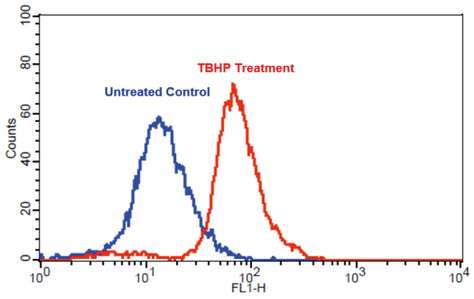

Figure 39. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22904). Cells were incubated with Amplite™ ROS Green at 37 ºC for 1 hour. Cells were then treated with (Red) or without (Blue) 100 µM TBHP at 37 ºC for 30 minutes. The fluorescence signals were monitored using a flow cytometer (BD FACSCalibur™) in FL1 channel.

Table 14. Intracellular Total ROS Activity Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
22903	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Deep Red Fluorescence*	200 tests	658	675
22900	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Green Fluorescence*	200 tests	492	520
22904	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	492	520
22902	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Orange Fluorescence*	200 tests	556	566
22901	Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit *Red Fluorescence*	200 tests	520	605

Thiol Detection

The monitoring of glutathione, its reduced (GSH) and oxidized (GSSG) states as well as their ratio (GSH/GSSG), in biological samples is essential for evaluating the redox and detoxification status of cells and tissues in relation to the protective role of glutathione against oxidative and free-radical-mediated cell injury. Cysteine metabolism disorders include cystinosis, an autosomal recessive disease produced by a defect in lysosomal transport, and cystinuria, a common heritable disorder of amino acid transport. Cysteine is unique among the amino acids found in proteins. There are a few reagents or assay kits available for quantifying thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols.

Amplite™ Fluorimetric Gluathione Assay Kit (Cat# 10055) and Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Kits (Cat# 10056 & 10060) are ultrasensitive fluorimetric assays used to quantify GSH and GSH/GSSG ratio, respectively. These kits use a proprietary non-fluorescent dye that becomes strongly fluorescent upon reacting with thiol. Amplite™ Fluorimetric Gluathione Assay Kit (Cat# 10055) provide a sensitive, one-step fluorimetric method to detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume.

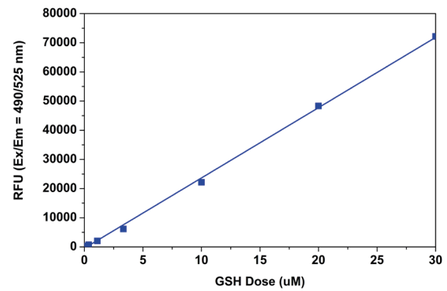

Figure 40. GSH dose responses were measured in a 96-well solid black plate with Amplite™ Fluorimetric Glutathione Assay Kit (Cat# 10055). As low as 10 nM (1 pmol/well) GSH was detected with 10 minutes incubation (n=3).

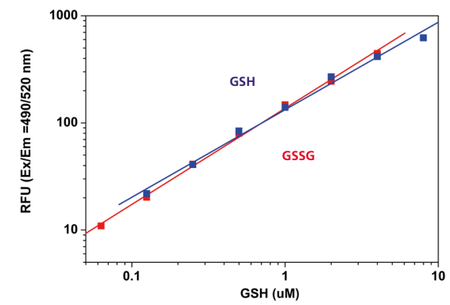

Figure 41. GSH and GSSG dose responses were measured with Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat#10056). Blue: GSH dose responses (0.063 µM to 4 µM); Red: GSSG dose responses (0.063 µM to 4 µM GSSG which is equivalent to 0.125 µM to 8 µM GSH).

The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 490/520 nm. For complex samples, we strongly recommend you use Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Kit (Cat# 10060) due to its much higher reproducibility and enhanced convenience.

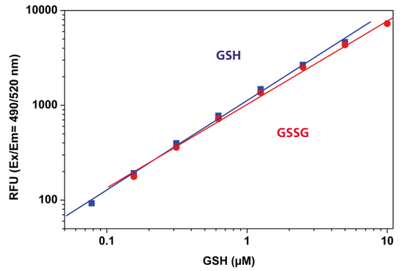

Figure 42. GSH and GSSG dose responses were measured with Amplite™ Rapid Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat# 10060). Blue: GSH dose responses (0.078 to 5 μM); Red: GSSG dose responses (0.078 to 5 μM GSSG which is equivalent to 0.156 to 10 μM GSH).

Table 15. Thiol Detection Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
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
10060	Amplite™ Rapid Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit *Green Fluorescence*	200 tests	510	524

Nitric Oxide Detection

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

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

Key Features of DAX-J2™ NO Detection Probes:

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

DAX-J2™ Red (Cat# 16301) is a non-fluorescent cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells under physiological conditions. Once inside the cell, the blocking groups on the DAX-J2™ reagent are released to generate a highly red fluorescent product upon NO oxidation. The DAX-J2™ fluorescent product can be detected using most flow cytometers and fluorescence microscopes equipped with the filter set of Texas Red®.

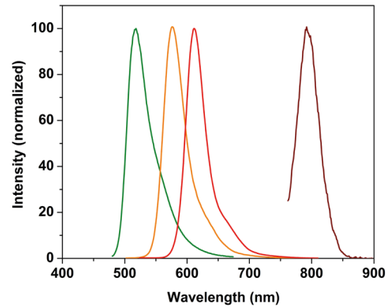

Figure 43. The spectral properties of DAX-J2™ reagents. DAF-2 (Green), DAX-J2™ Orange (Orange, Cat# 16300), Red (Red, Cat# 16301) and IR (Dark Red, Cat# 16302) in PBS buffer (pH 7.2).

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

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

DAX-J2™ Ratio 580/460 (Cat# 16310) is a new nitric oxide (NO) sensor recently developed by AAT Bioquest. It is a cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells in a ratiometric mode. Once inside the cell, the blocking groups on the DAX-J2™ reagent are released to induce fluorescence ratio changes at wavelengths of 580 nm and 460 nm upon NO oxidation. The fluorescence intensities at 580 nm and 460 nm can be detected using the filter sets of Cy3®/TRITC and BD Horizon™ V450/ Pacific Blue. Most of flow cytometers and fluorescence microscopes

are equipped with these two filter sets. DAX-J2™ Ratio 580/460 has distinct advantages for NO detection over the popular DAF-2 NO probe: 1) DAX-J2™ Ratio 580/460 does not require esterase activity for NO detection. DAF-2 requires intracellular esterases to cleave its acetate groups for detecting NO activity. 2) DAX-J2™ product exhibits pH-independent fluorescence while DAF-2 has its fluorescence highly affected by pH. 3) DAX-J2™ Ratio 580/460 can be monitored in a ratiometric mode.

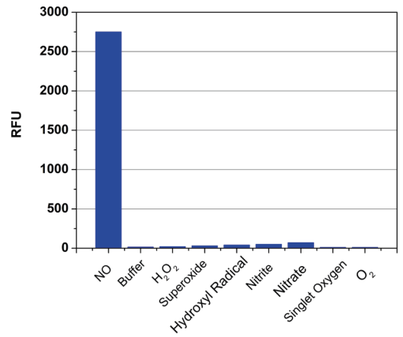

Figure 44. Fluorescence responses of DAX-J2™ Orange (5 μM, Cat# 16300) to different reactive oxygen species (1 mM) in PBS buffer (pH 7.2). The fluorescence intensities were measured at Ex/Em = 540/570 nm.

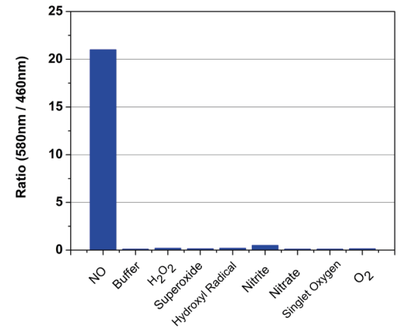

Figure 45. Fluorescence responses of DAX-J2™ Ratio 580/460 (2 μM, Cat# 16310) to different reactive oxygen species (1 mM) in PBS buffer (pH = 7.2). The fluorescence intensities were measured at 580 nm and 460 nm respectively.

Table 16. Multicolor Nitric Oxide (NO) Probes

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

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

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

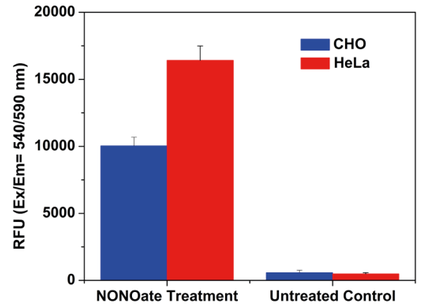

Figure 46. Detection of exogenous nitric oxide (NO) in cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16350). CHO-K1 and HeLa cells were seeded at 50,000 cells/well/100 μL overnight in a 96-well black wall/clear bottom plate. Cells were incubated with Nitrixyte™ Orange working solution at 37 °C for 30 minutes. The cells were treated with or without 1mM DEA NONOate at 37 °C for 30 minutes. The fluorescence signals were monitored at Ex/Em = 540/590 nm (cut off at 570 nm) with bottom read mode.

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

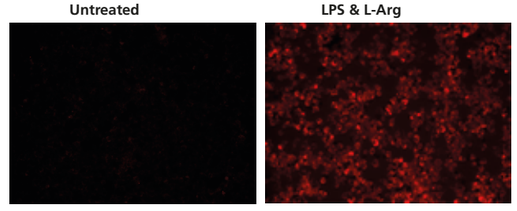

Figure 47. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16350). Raw 264.7 cells were seeded at 100,000 cells/well/100 μL overnight in a 96-well black wall/clear bottom plate. Cells were incubated with Nitrixyte™ Orange, and treated with (Bottom) or without (Top) 20 μg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) at 37 °C for 16 hours. The fluorescence signals were measured using fluorescence microscope with a TRITC filter.

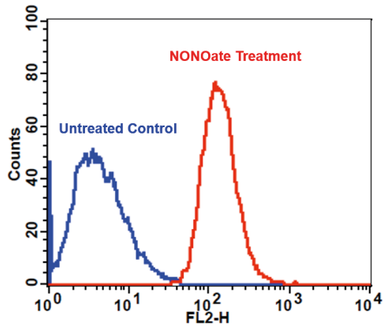

Figure 48. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16351). Cells were incubated with Nitrixyte™ Orange at 37 °C for 30 minutes and washed twice with assay buffer. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 30 minutes. The fluorescence signals were monitored in FL2 channel.

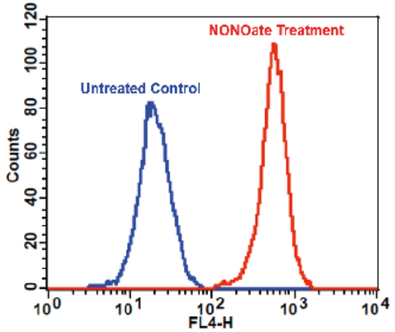

Figure 49. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat# 16356). Cells were incubated with Nitrixyte™ Red at 37 °C for 30 minutes. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 2 hours. The fluorescence signals were monitored using a flow cytometer (BD FACSCalibur™) in FL4 channel.

Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16359) uses Nitrixyte™ NIR that can react with NO to generate strong near-infrared (NIR) fluorescence signal. Nitrixyte™ NIR can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy5® or APC. This kit is optimized for fluorescence imaging and microplate reader applications. Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16360) is optimized for flow cytometry applications.

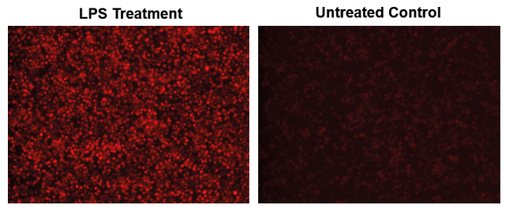

Figure 50. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat#16359). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. Cells were co-incubated with Nitrixyte™ NIR, with or without 20 µg/mL of lipopolysaccharide (LPS) and 1 mM L-Arginine (L-Arg) in cell culture medium at 37 °C for 16 hours. The solution in each well was removed, and Assay Buffer II was added before fluorescence measurement. The fluorescence signal was measured using fluorescence microscope with a Cy5® filter.

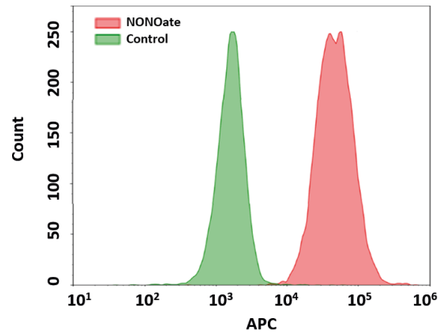

Figure 51. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat#16360). Cells were incubated with Nitrixyte™ NIR at 37 °C, 5% CO_2 for 30 minutes. Spin down and wash cells with Hanks and 10 mM HEPES buffer. Cells were further treated with or without 1 mM DEA NONOate in Assay Buffer (Component C) at 37 °C, 5% CO_2 incubator for 60 minutes.

Table 17. Intracellular Nitric Oxide (NO) Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
16360	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *NIR Fluorescence Optimized for Flow Cytometry*	100 tests	640	675
16359	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *NIR Fluorescence Optimized for Microplate Reader*	200 tests	650	680
16351	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	100 tests	545	576
16350	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Orange Fluorescence Optimized for Microplate Reader*	200 tests	545	576
16356	Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests	588	610

Peroxynitrite Assays

Peroxynitrite ($ONOO^-$) is a strong oxidizing species and a highly active nitrating agent. Peroxynitrite is formed from the reaction between superoxide radicals and nitric oxide generated in cells. It can cause damages to a wide array of biomolecules including proteins, enzymes, lipids and nucleic acids, eventually contributing to cell death. Meanwhile, peroxynitrite can also have protective activities in vivo by contributing to host-defense responses against invading pathogens. Therefore, peroxynitrite is an essential biological oxidant involved in a board range of physiological and pathological processes. Due to its extremely short half-life and low steady-state concentration, it has been challenging to detect and understand the role of peroxynitrite in biological systems.

Amplite™ Fluorimetric Peroxynitrite Quantification Kit (Cat# 16316) has been developed to address this unmet need. provides a sensitive tool to measure ONOO- in solution. DAX-J2™ PON Green 99 reacts with ONOO- to generate a bright green fluorescent product. It specifically reacts with ONOO- with high selectivity over other reactive oxygen species (ROS) and reactive nitrogen species (RNS). This kit can be used with a fluorescence microplate reader and spectrometer.

Cell Meter™ Fluorimetric Intracellular Peroxynitrite ($ONOO^-$) Assay Kits (Cat# 16315 & 16317) provides a sensitive tool to monitor $ONOO^-$ level in living cells. AAT Bioquest's DAX-J2™ PON Green is developed as an excellent fluorescent probe, which can specifically react with intercellular $ONOO^-$ to generate a bright green fluorescent product. Kit 16315 can be used in fluorescence imaging and fluorescence microplate reader. Kit 16317 is optimized for flow cytometry.

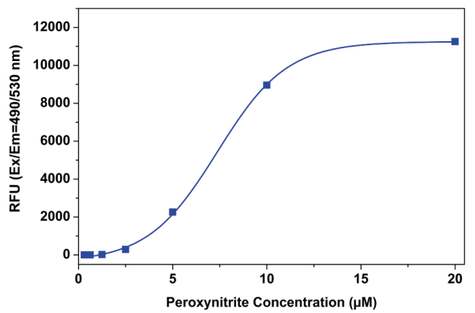

Figure 52. Peroxynitrite was measured with the Amplite™ Fluorimetric Peroxynitrite Quantification Kit (Cat# 16316) on a solid black 96-well plate using a Gemini microplate reader. As low as 1.25 µM was detected. (Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.)

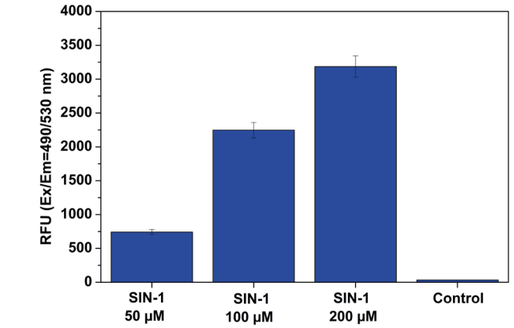

Figure 53. Detection of peroxynitrite in living cells upon SIN-1 treatment using Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16315). RAW 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. Cells were co-incubated with DAX-J2™ PON Green working solution and SIN-1 at the concentration from 50 to 200 µM at 37 °C for 1 hour. Cells incubated with DAX-J2™ PON Green without SIN-1 treatment were used as control. The fluorescence signal were monitored at Ex/Em = 490/530 nm (cut off = 515 nm) with bottom read mode using a FlexStation® microplate reader (Molecular Devices).

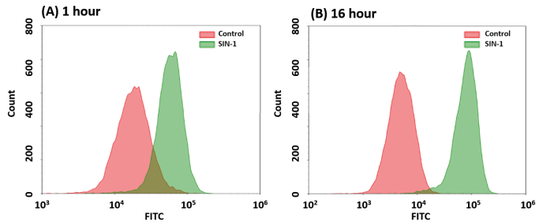

Figure 54. Detection of peroxynitrite in Jurkat cells upon SIN-1 treatment using AAT Bioquest's Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16317). (A) Jurkat cells were co-incubated with DAX-J2™ PON Green and 200 µM SIN-1 in full medium at 37 °C for 1 hour. (B) Cells were stained with DAX-J2™ PON Green for 1 hour, washed with PBS and then incubated with 200 µM SIN-1 in full medium at 37 °C for 16 hours. Cells stained with DAX-J2™ PON Green without SIN-1 treatment were used as a control. Fluorescence intensity was measured using ACEA NovoCyte® flow cytometer in FITC channel.

Table 18. Peroxynitrite Assay Kits

Cat #	Product Name	Unit Size	Ex (nm)	Em (nm)
16316	Amplite™ Fluorimetric Peroxynitrite Quantification Kit *Green Fluorescence*	100 tests	502	535
16315	Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit *Green Fluorescence*	100 tests	502	535
16317	Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit *Optimized for Flow Cytometry*	100 tests	588	610

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Unless otherwise specified, all products are for Research Use Only.
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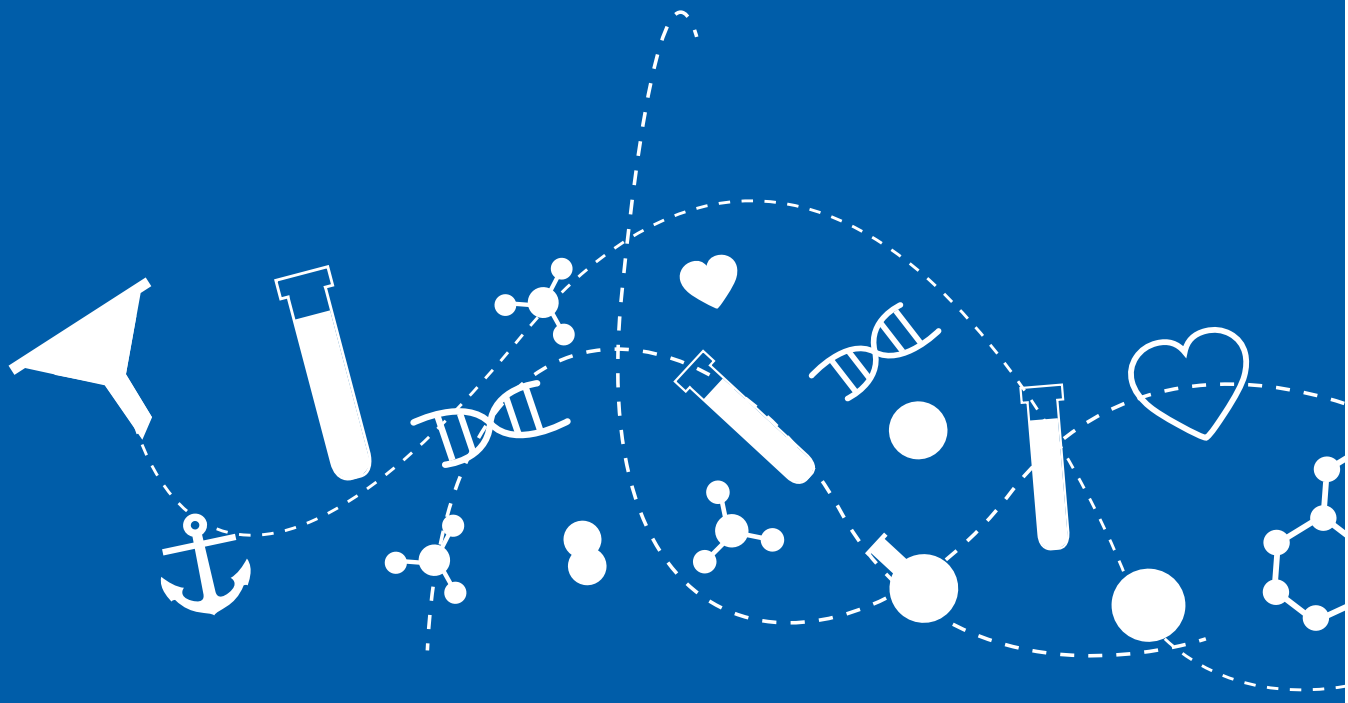

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WIR HABEN DIE SUBSTANZ.

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