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AssayWise Letters

A Special Issue for Reactive Oxygen Species Detection

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Advancing Assay & Test Technologies

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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 non-fluorescent Amplite™ Red peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. It provides a sensitive, one-step fluorimetric assay to detect as 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 at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~570 nm.

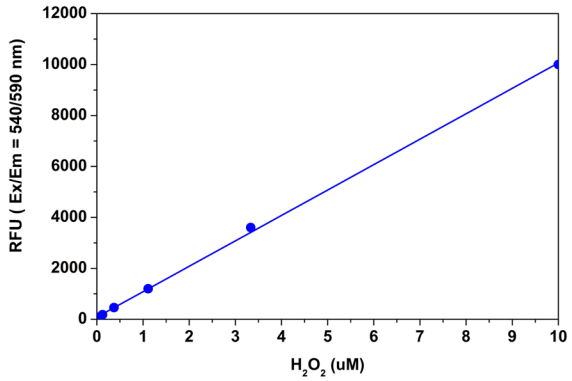

Figure 1. H_2O_2 dose responses were measured in a 384-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501). As low as 0.03 µM H_2O_2 was detected with 30 minutes incubation (n=3).

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 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).

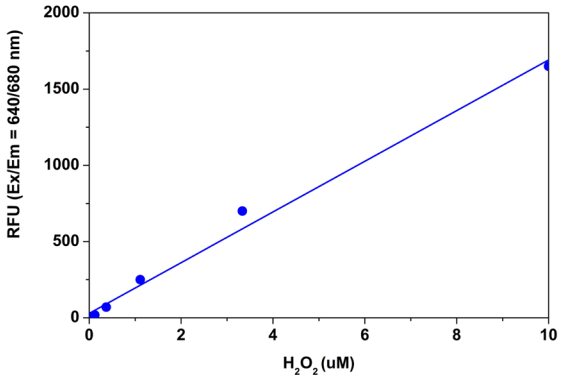

Figure 2. H_2O_2 dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502). As low as 0.03 µM H_2O_2 was detected.

Table 1. Hydrogen Peroxide Assay Kits

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

Intracellular Hydrogen Peroxide Detection

Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503) uses our unique ROS Green™ to quantify hydrogen peroxide in live cells. Cell-permeable ROS 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

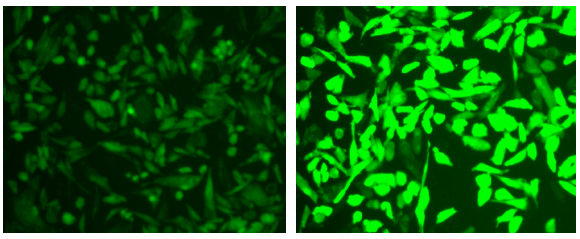

Figure 3. Images of live CHO-K1 cells in a 96-well plate. Live CHO-K1 cells were stained with Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11503). Left: Control cells. Right: Cells treated with 100 µM H_2O_2 at room temperature for 5 minutes.

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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 microscopy.

Dihydrofluorescein diacetate (also called fluorescein diacetate, Cat# 15203) is hydrolyzed by cellular esterases to dihydrofluorescein (also called fluorescein), 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 oxidant 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.

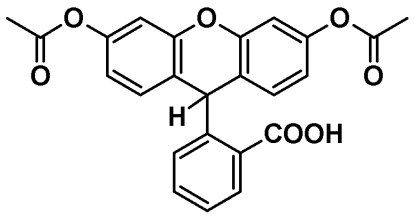

Figure 4. The chemical structure of Dihydrofluorescein Diacetate (Cat# 15203).

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 2. Intracellular Hydrogen Peroxide Detection Assay and Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
11503	Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*	200 tests	492	515
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.

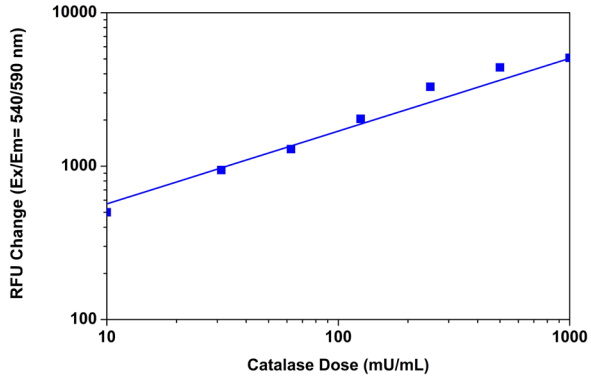

Figure 5. Catalase does responses were measured with Amplite™ Fluorimetric Catalase Assay Kit (Cat# 11306) in a 96-well solid black plate using a Gemini fluorescence microplate reader. As low as 30 mU/mL catalase was detected with 30 minutes incubation (n=3).

Table 3. Catalase Detection Assay Kit

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

Peroxidase Detection

Peroxidase is a small molecule (MW ~40 KD) that can usually be conjugated to an antibody. Due to its small size, it rarely causes steric hindrance problem 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.

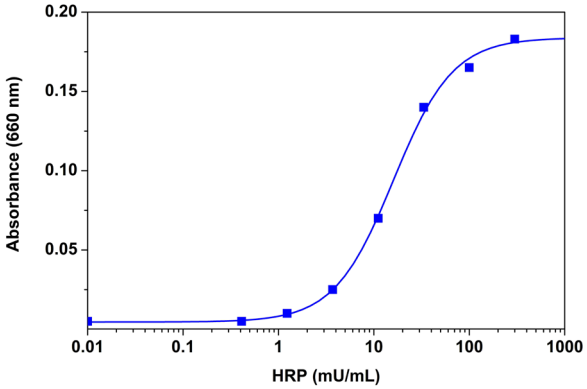

Figure 6. HRP does responses were measured with Amplite Colorimetric Peroxidase Assay Kit (Cat# 11551) in a 96-well white wall/clear bottom plate. As low as 3 mU/mL peroxidase was detected.

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, etc. The kits provide an optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments. Kit 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 ws 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.

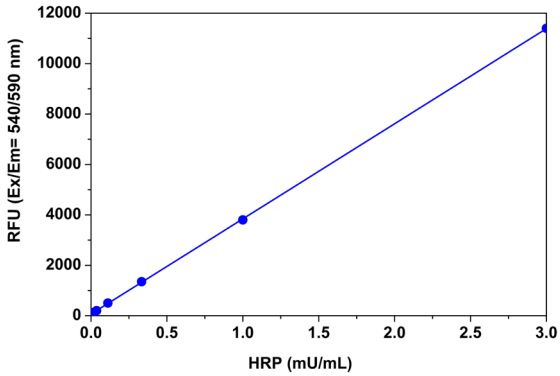

Figure 7. HRP does responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11552) in a 384-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 µU/mL peroxidase was detected with 30 minutes incubation (n=3).

Kit 11553 uses Amplite™ IR, our near infrared flurogenic 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 Kit 11553.

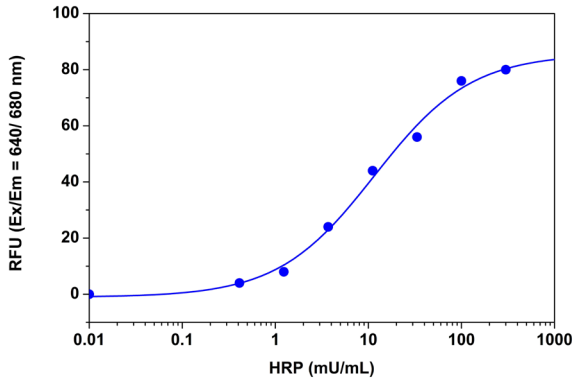

Figure 8. HRP does responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553) in a solid black 384-well plate using a Gemini fluorescence microplate reader. As low as 1 mU/mL peroxidase was detected with 30 minutes incubation (n=3).

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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 to immune deficiency. There are considerable interests 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.

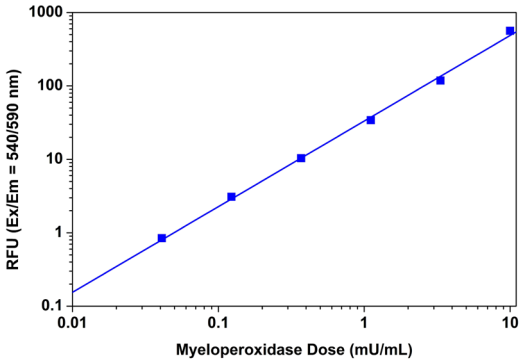

Figure 9. Myeloperoxidase does responses were measured with Amplite™ Fluorimetric Myeloperoxidase Assay Kit (Cat# 11301) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.1 mU/mL myeloperoxidase was detected with 60 minutes incubation (n=3).

Amplite™ ADHP (Cat# 11000) is chemically the same 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, etc.

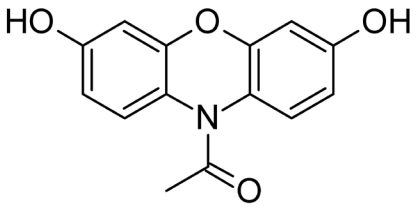

Figure 10. The chemical structure of Amplite™ ADHP (Cat# 11000).

Horseradish peroxidase (HRP) and HRP conjugates facilitate the ABTS oxidation in the presence of hydrogen peroxide, turning ABTS into its blue-green oxidized product. This chromogenic reaction is widely used for quantify HRP in ELISA assays. The oxidized ABTS product has the 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 reaction done 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.

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 ultrasensitive quantitative substrate system.

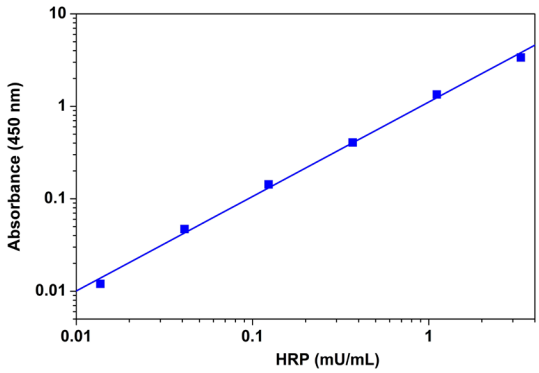

Figure 11. HRP does responses were measured with ReadiUse™ TMB Substrate Solution (Cat# 11003) in a clear 96-well plate. As low as 3 µU/well peroxidase was detected with 10 minutes incubation.

Amplite™ Red (Cat# 11011) is a sensitive fluorogenic peroxidase substrate generating a highly red fluorescent product that has maximum absorption at 571 nm and maximum emission at 585 nm. Unlike other HRP substrates, such as dihydrofluoresceins and dihydrorhodamines, Amplite™ Red has minimum air-oxidation. Amplite™ Red is one of the most sensitive and stable fluorogenic probes for detecting HRP and H_2O_2. Amplite™ Red has been widely used to detect HRP in many immunoassays. On the other hand, Amplite™ Red can also be used to detect trace amount of H_2O_2. The Amplite™ Red-based H_2O_2 detection is at least one order of magnitude more sensitive than the commonly used scopoletin assays for H_2O_2. Because H_2O_2 is produced in many enzymatic redox reactions, Amplite™ Red 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, etc.

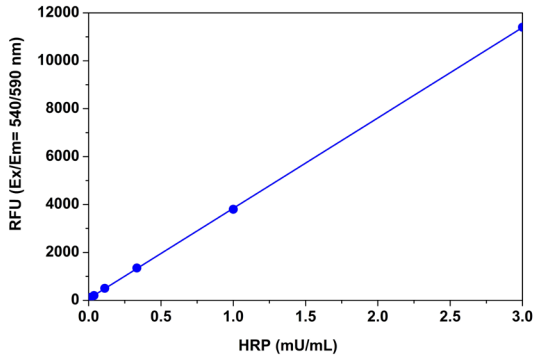

Figure 12. HRP does responses were measured with Amplite™ Red (Cat# 11011). As low as 10 µU/mL peroxidase was detected with 30 minutes incubation (n=3).

Table 4. Peroxidase Detection Assay Kits and Probes

Cat #	Product Name	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
11011	Amplite™ Red	100 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

Hydroxyl Radical Detection

The detection of intracellular hydroxyl radical is of central importance to the understanding of proper cellular redox regulation and the impact of its dysregulation on various pathologies. The hydroxyl radical (•OH) is one of the reactive oxygen species (ROS) that are highly reactive with other molecules to achieve stability. In general, hydroxyl radical is considered to be a harmful by-product 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.

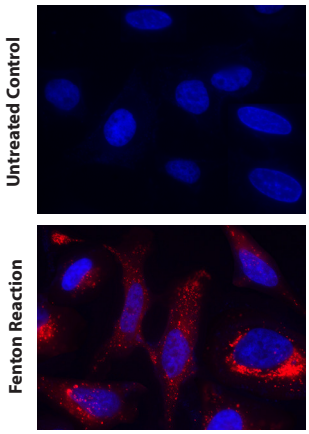

Figure 13. Fluorescence images of hydroxyl radical measurements in HeLa cells using Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit (Cat# 16055). Control (Top): HeLa cells were kept in 1X HBSS buffer without treatment. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530). Fenton Reaction (Bottom): Cells were treated with 10 µM $CuCl_2$ and 100 µM H_2O_2 in 1X HBSS buffer at 37 °C for 1 hour.

Table 5. Hydroxyl Radical Assay Kit

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

Superoxide Detection

Hydroethidine (Cat# 15200), a redox-sensitive probe, has been widely used to detect intracellular superoxide anion. 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 a nd 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.

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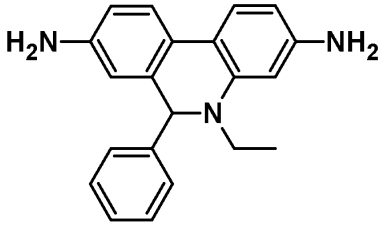

Figure 14. The chemical structure of Hydroethidine (Cat# 15200).

Table 6. Superoxide Detection Probe

Cat #	Product Name	Size	Ex (nm)	Em (nm)
15200	Hydroethidine	25 mg	518	605

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.

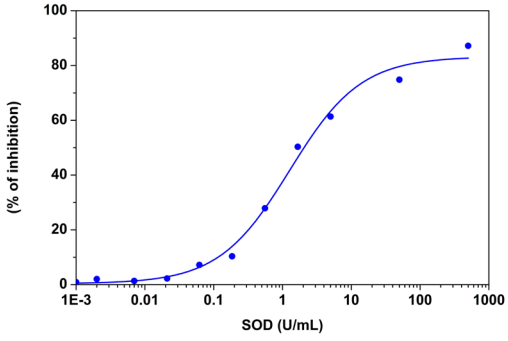

Figure 15. SOD dose responses were measured with Amplite™ Colorimetric Superoxide Dismutase Assay Kit (Cat# 11305). As low as 0.1 U/mL SOD was detected with 60 minutes incubation (n=3).

Table 7. Superoxide Dismutase Detection Assay Kit

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

Xanthine Assay

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

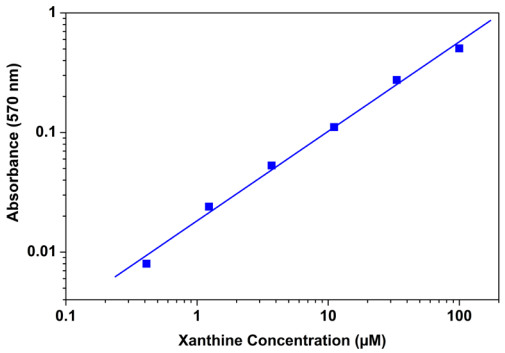

Figure 16. Xanthine dose responses were measured with Amplite™ Colorimetric Xanthine Assay Kit (Cat# 13842) in a 96-well clear bottom plate. As low as 1.2 μM xanthine was detected with 30 minutes incubation (n=3).

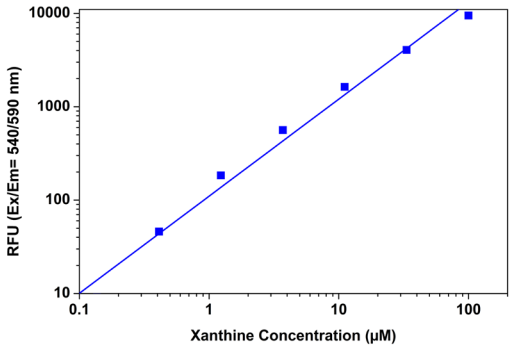

Figure 17. Xanthine dose responses were measured with Amplite™ Fluorimetric Xanthine Assay Kit (Cat# 13843) in a 96-well black solid plate. As low as 0.4 μM xanthine was detected with 30 minutes incubation (n=3).

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 0.4 µM xanthine was detected in a 100 µL reaction volume.

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™ Fluorimetric Xanthine Oxidase Assay Kit (Cat# 11304) provides 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 kit uses 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 Amplite™ Xanthine Oxidase Assay Kit, as low as 0.15 mU/mL xanthine oxidase was detected in a 100 µL reaction volume.

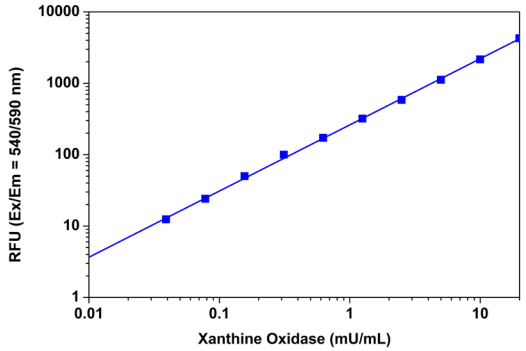

Figure 18. Xanthine oxidase dose responses were measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit (Cat# 11304) in a 96-well black solid plate. As low as 0.15 mU/mL xanthine oxidase was detected with 60 minutes incubation (n=3).

Table 8. Xanthine Detection Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
13842	Amplite™ Colorimetric Xanthine Assay Kit	200 tests	575	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 Assay

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.

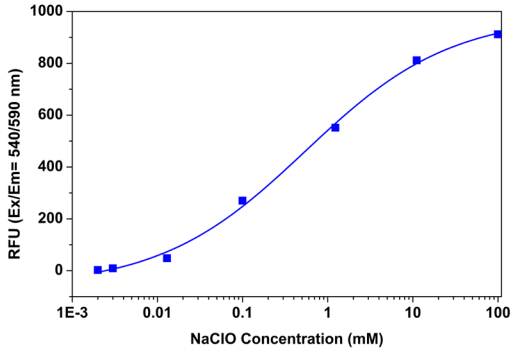

Figure 19. Hypochlorite was measured with Amplite™ Fluorimetric Hypochlorite (Hypochlorous Acid) Assay Kit (Cat# 13846). As low as 0.003 mM (~3 µM) sodium hypochlorite (NaClO) was detected with 10-30 minutes incubation (n=3).

Table 9. Hypochlorite Assay Kits

Cat #	Product Name	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

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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 to both hydroxyl radical and supperoxide.

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

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.

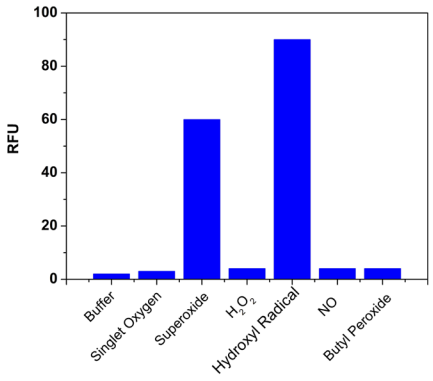

Figure 20. The responses of ROS Brite™ 570 (Cat# 16000) to different ROS species.

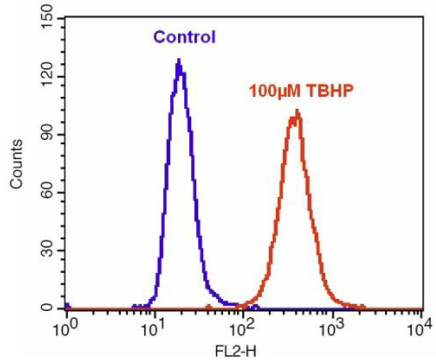

Figure 21. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100 μM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and then loaded with ROS Brite™ 570 (Cat# 16000) in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using FL2 channel.

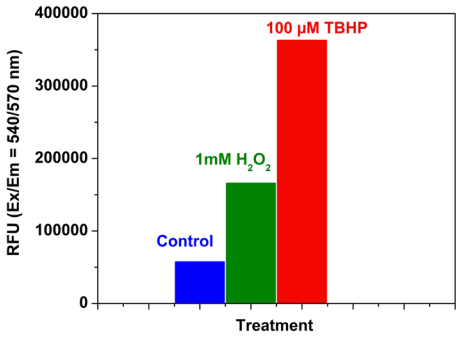

Figure 22. Detection of ROS in HeLa cells. The cells were untreated (control) or treated with 1 mM H_2O_2 or 100 μM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. ROS Brite™ 570 (Cat# 16000) (100 μL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence signals were monitored at Ex/Em = 540/570 nm (cut off at 550 nm) with bottom read mode.

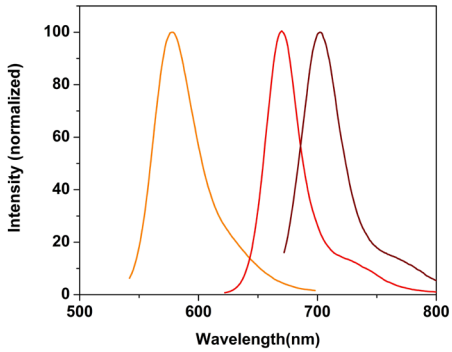

Figure 23. The fluorescence spectra of ROS Brite™ 570 (Yellow, Cat# 16000), ROS Brite™ 670 (Orange, Cat# 16002) and ROS Brite™ 700 (Red, Cat# 16004).

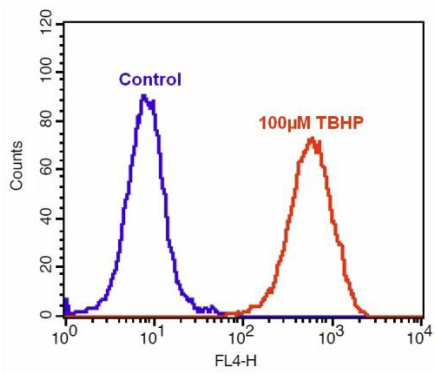

Figure 24. Detection of ROS in Jurkat cells. Jurkat cells were untreated (Blue) or treated with 100 μM tert-butyl hydroperoxide (TBHP) (Red) for 30 minutes at 37 °C, and loaded with ROS Brite™ 670 (Cat# 16002) for 1 hour. The fluorescence intensities were measured with a FACSCalibur™ flow cytometer using 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 oxidant stress. This probe can be conveniently used to monitor cellular redox processes for multiplexing assays with FITC-labeled antibodies or GFP cell lines. The oxidized product is highly fluorescent in cells. ROS

Brite™ DHCF provides a valuable tool for investigating oxidative stress in various pathologies.

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.

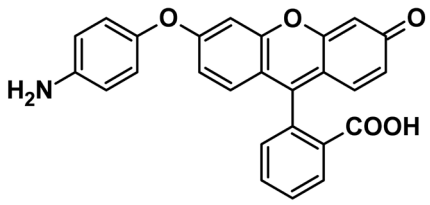

Figure 25. The chemical structure of ROS Brite™ APF (Cat# 16050).

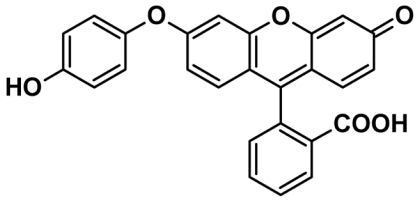

Figure 26. The chemical structure of ROS Brite™ HPF (Cat# 16051).

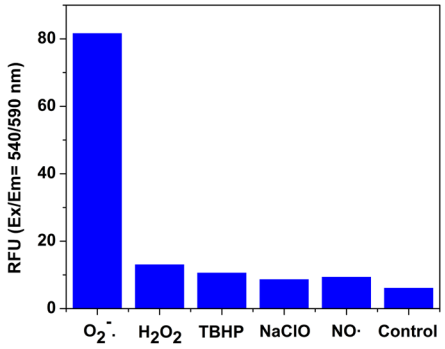

Figure 27. Fluorescence response of MitoROS™ 580 (10 μM, Cat# 16052) to different reactive oxygen species (ROS) and reactive nitrogen species (RNS). The fluorescence intensities were monitored at Ex/Em = 540/590 nm.

MitoROS™ 580 (Cat# 16052) is a superoxide-sensitive dye that is localized 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.

Table 10. Total ROS Activity Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
16052	MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS)*	500 tests	510	580
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 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 (Cat# 22900, 22901, 22902 & 22903) are in an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. Kit 22904 is optimized for flow cytometry applications, its signal can be detected at Ex/Em = 490/520 nm (FL1 channel).

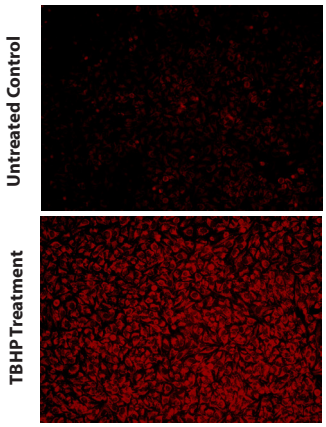

Figure 28. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22902) in a Costar 96-well black wall/clear bottom plate. Top: Untreated control cells. Bottom: Cells treated with 100 μM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

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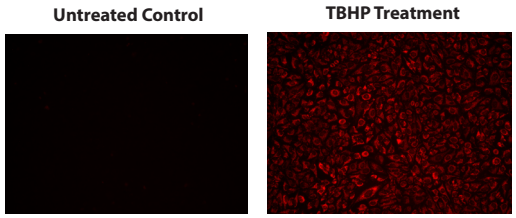

Figure 29. Images of HeLa cells stained with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22903) in a Costar 96-well black wall/clear bottom plate. Left: Untreated control cells. Right: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes before staining.

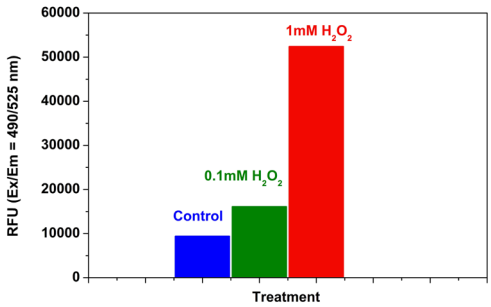

Figure 30. Detection of ROS in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22900). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was 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.

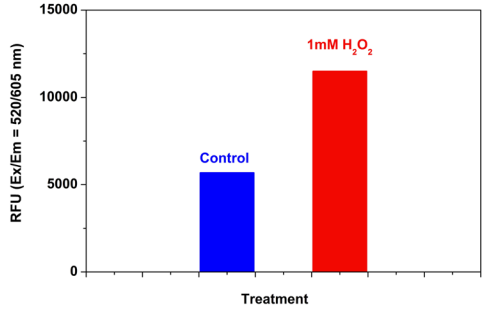

Figure 31. Detection of ROS in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22901). Jurkat cells were seeded on the same day at 300,000 cells/100 µL/well in a 96-well black wall/clear bottom plate. The ROS assay loading solution (100 µL/well) was added 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.

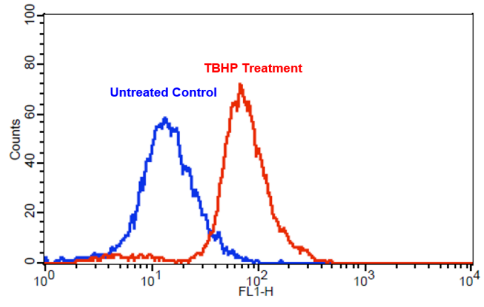

Figure 32. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat# 22904). Cells were incubated with Amplite™ ROS Green at 37 ºC for 1 hour. Cells were then treated with (Red) or without (Blue) 100 µM TBHP at 37 ºC for 30 minutes. The fluorescence signals were monitored using a flow cytometer (BD FACSCalibur™) in FL1 channel.

Table 11. Intracellular Total ROS Activity Assay Kits

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

Thiol Detection

The monitoring of reduced and oxidized glutathione (GSH) 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 quantitating thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols.

Amplite™ Fluorimetric Glutathione Assay Kit (Cat# 10055) provides an ultrasensitive fluorimetric assay to quantify GSH in sample. The kit uses a proprietary non-fluorescent dye that becomes strongly fluorescent upon reacting with thiol. The kit provides a sensitive, one-step fluorimetric method to detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 490/520 nm.

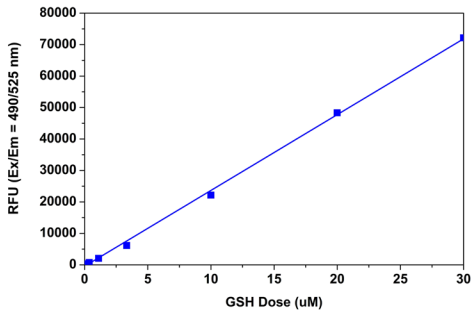

Figure 33. GSH dose responses were measured in a 96-well solid black plate with Amplite™ Fluorimetric Glutathione Assay Kit (Cat# 10055). As low as 10 nM (1 pmol/well) GSH was detected with 10 minutes incubation (n=3).

The monitoring of GSH/GSSG ratio and the quantification of GSSG in biological samples are 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. There are a few reagents or assay kits available for the quantitation of thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols.

Amplite™ Fluorimetric Glutathione GSH/GSSG Ratio Kits (Cat# 10056 & 10060) provide robust tools to quantify GSH. The kits use proprietary non-fluorescent dyes that become strongly fluorescent upon reacting with thiol. These kits provide a sensitive, one-step fluorimetric method to detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. For complex samples, we strongly recommend you use Kit 10060 due to its much higher reproducibility and enhanced convenience.

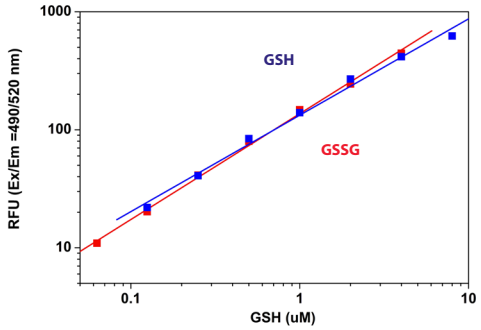

Figure 34. GSH and GSSG dose responses were measured with Amplite Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat#10056). Blue: GSH dose responses (0.063 µM to 4 µM); Red: GSSG dose responses (0.063 µM to 4 µM GSSG which is equivalent to 0.125 µM to 8 µM GSH).

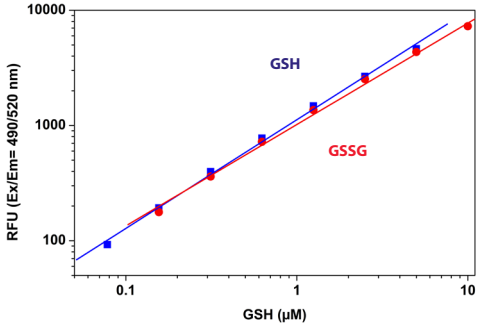

Figure 35. GSH and GSSG dose responses were measured with Amplite™ Rapid Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit (Cat# 10060). Blue: GSH dose responses (0.078 to 5 µM); Red: GSSG dose responses (0.078 to 5 µM GSSG which is equivalent to 0.156 to 10 µM GSH).

Table 12. Thiol Detection Assay Kits

Cat #	Product Name	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. Nitric oxide is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals, such as nitroglycerine and amyl nitrite, were discovered, more than a century after their first use in medicine, to be active through the mechanism of being precursors to nitric oxide. Low levels of nitric oxide production are important in protecting organs, such as the liver, from ischemic damage.

Key Features of DAX-J2™ NO Detection Probes:

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

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

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

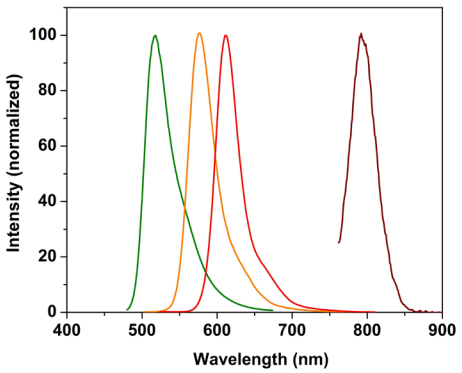

Figure 36. The spectral properties of DAX-J2™ reagents. DAF-2 (Green), DAX-J2™ Orange (Orange, Cat# 16300), Red (Red, Cat# 16301) and IR (Dark Red, Cat# 16302) in PBS buffer (pH 7.2).

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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.

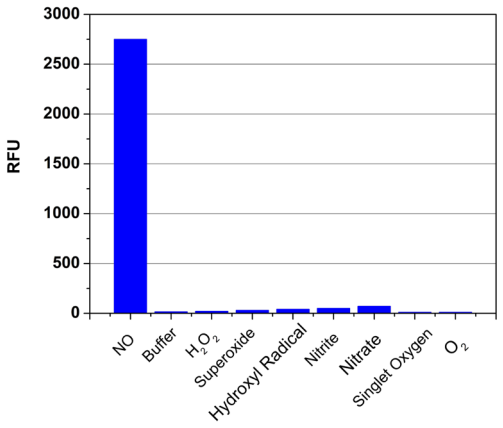

Figure 37. Fluorescence responses of DAX-J2™ Orange (5 μM, Cat# 16300) to different reactive oxygen species (1 mM) in PBS buffer (pH 7.2). The fluorescence intensities were measured at Ex/Em = 540/570 nm.

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

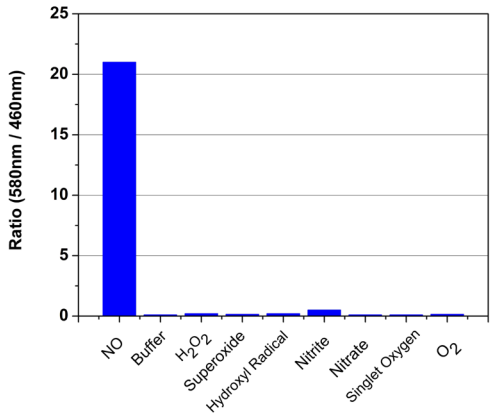

Figure 38. Fluorescence responses of DAX-J2™ Ratio 580/460 (2 μM, Cat# 16310) to different reactive oxygen species (1 mM) in PBS buffer (pH = 7.2). The fluorescence intensities were measured at 580 nm and 460 nm respectively.

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.

Table 13. Multicolor Nitric Oxide (NO) Probes

Cat #	Product Name	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

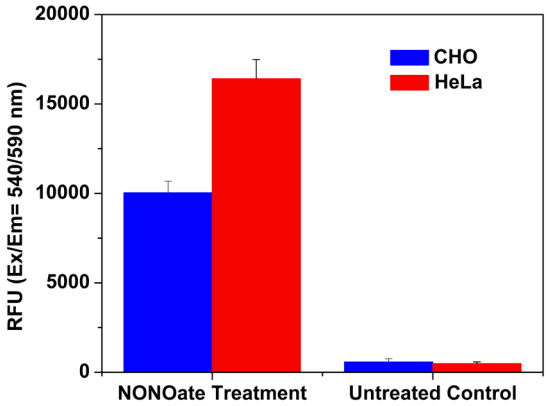

Figure 39. Detection of exogenous nitric oxide (NO) in cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat#16350). CHO-K1 and HeLa cells were seeded at 50,000 cells/well/100 μL overnight in a 96-well black wall/clear bottom plate. Cells 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.

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.

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.

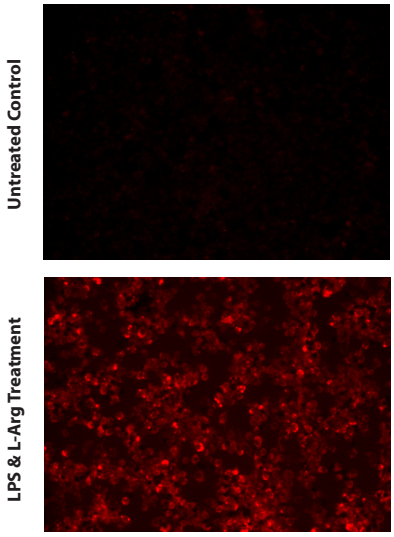

Figure 40. Fluorescence images of endogenous nitric oxide (NO) measurement in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16350). Raw 264.7 cells were seeded at 100,000 cells/well/100 µL overnight in a 96-well black wall/clear bottom plate. Cells 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.

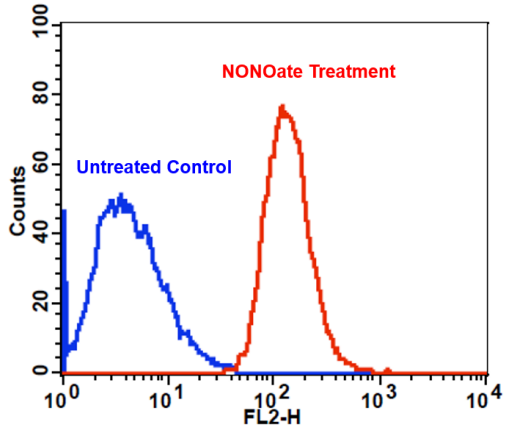

Figure 41. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Assay Kit (Cat# 16351). Cells were incubated with Nitrixyte™ Orange at 37 °C for 30 minutes and washed twice with assay buffer. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 30 minutes. The fluorescence signals were monitored in FL2 channel.

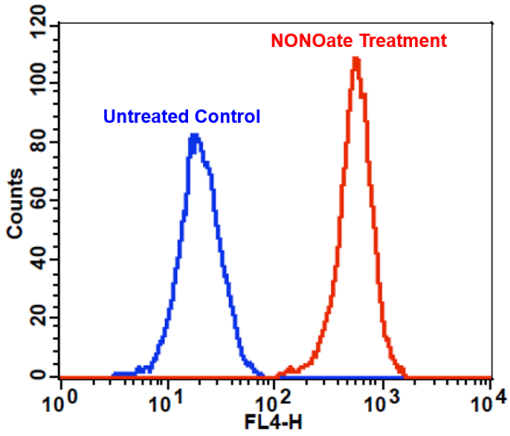

Figure 42. Detection of exogenous nitric oxide (NO) in Jurkat cells upon DEA NONOate treatment (NO donor) using Cell Meter™ Fluorimetric Intracellular Nitric Oxide Activity Assay Kit (Cat# 16356). Cells were incubated with Nitrixyte™ Red at 37 °C for 30 minutes. The cells were treated with (Red) or without (Blue) 1mM DEA NONOate at 37 °C for 2 hours. The fluorescence signals were monitored using a flow cytometer (BD FACSCalibur™) in FL4 channel.

Table 14. Intracellular Nitric Oxide (NO) Assay Kits

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

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