Datasheet for ABIN2345004

OxiSelect™ Hydrogen Peroxide / Peroxidase Assay Kit (Fluorometric)

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Overview

Quantity:	500 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit is a sensitive quantitative fluorometric assay for hydrogen peroxide or peroxidase.
Brand:	OxiSelect™
Sample Type:	Cell Lysate, Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Sensitivity:	50 nM
Characteristics:	OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit is a simple HTS-compatible assay for measuring hydrogen peroxide concentrations or peroxidase activities in biological samples without any need for pretreatment. In the presence of H2O2 and horseradish peroxidase (HRP), non-fluorescent ADHP (10-Acetyl-3, 7-dihydroxyphenoxazine) is oxidized to the highly fluorescent Resorufin. The probe has less background and greater stability. The ADHP-based H2O2 detection is at least one order of magnitude more sensitive than the commonly used Xylenol Orange (FOX) colorimetric assay for H2O2. The probe can be also used as an ultrasensitive assay for peroxidase activity when H2O2 is in excess. The kit has a detection sensitivity limit of 50 nM (H2O2) or 0.1 mU/mL (Peroxidase). Each kit provides sufficient reagents to perform up to 500 assays, including standard curve and unknown samples. ADHP

Product Details

	(10-Acetyl-3, 7-dihydroxyphenoxazine)
Components:	1. ADHP Probe : One 250 µL amber tube of a 10 mM solution in DMSO. 2. HRP : One 100 µL tube of a 100 U/mL solution in glycerol*. 3. Hydrogen Peroxide : One 100 µL amber tube of an 8.8 M solution. 4. 10X Assay Buffer : One 25 mL bottle. *Note: One unit is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 and 20°C.
Material not included:	1. Distilled or deionized water 2. 1X PBS for sample dilutions 3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 5. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate 6. Multichannel micropipette reservoir 7. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range. 3

Target Details

Background:	Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. Research has shown that excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. Peroxides, such as hydrogen peroxide (H2O2), are some of the most well documented ROS produced under oxidative stress conditions. Hydrogen peroxide is an ROS that is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. Hydrogen peroxide is poisonous to eukaryotic cells and in high doses can initiate oxidation of DNA, lipids, and proteins, which can lead to mutagenesis and cell death. The cellular damage caused by peroxides have been implicated in the development of many pathological conditions, such as ageing, asthma, arthritis, diabetes, cardiovascular disease, atherosclerosis, Down's Syndrome, and neurodegenerative diseases.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detect hydrogen peroxide concentrations as low as 50 nM • Suitable for use with cell lysates, tissue homogenates, cell culture supernatants, plasma, serum, urine, or other biological fluids • Simple assay protocol provides results in 30-90 minutes, depending on sample type
Protocol:	In the presence of HRP, ADHP reacts with H2O2 in a 1:1 stoichiometry to produce highly

	fluorescent Resorufin. The Resorufin product can be easily read by a fluorescence microplate reader with an excitation of 530-560 nm and an emission of 590 nm. Fluorescence values are proportional to the H2O2 or peroxidase levels within the samples. The H2O2 or peroxidase content in unknown samples is determined by comparison with its respective standard curve.
Reagent Preparation:	Note: All reagents must be brought to room temperature prior to use. • 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. • ADHP/HRP Working Solution (Hydrogen Peroxide Assay): If measuring hydrogen peroxide, prepare an ADHP/HRP Working Solution by adding ADHP to a final concentration of 100 µM and HRP to a final concentration of 0.2 U/mL in 1X Assay Buffer (eg. Add 50 µL ADHP stock solution and 10 µL HRP stock solution to 4.940 mL 1X Assay Buffer). This volume is enough for ~100 assays. The ADHP/HRP Working Solution is stable for 1 day. Prepare only enough for immediate use. • ADHP/H2O2 Working Solution (Peroxidase Assay): If measuring peroxidases, prepare the ADHP/H2O2 Working Solution by adding ADHP to a final concentration of 100 µM and H2O2 to a final concentration of 2 mM in 1X Assay Buffer. First perform a 1:1000 dilution of the stock H2O2 in 1X Assay Buffer. Use only enough for immediate applications (eg. Add 5 µL of H2O2 to 4.995 mL 1X Assay Buffer). This solution has a concentration of 8.8 mM. Use this 8.8 mM H2O2 solution to prepare a 2 mM H2O2 solution in ADHP/1X Assay Buffer (eg. Add 50 µL ADHP stock solution and 1.14 mL of the prepared 8.8 mM H2O2 solution to 3.81 mL 1X Assay Buffer). This volume is enough for ~100 assays. The Working Solution is stable for 1 day. Prepare only enough for immediate use.
Sample Preparation:	• Cell culture supernatant: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the H2O2 standard curve in the same non-conditioned media. Serum should be avoided, as it interferes with the assay. Note: Maintain pH between 7 and 8 for optimal working conditions as the ADHP is unstable at high pH (>8.5). • Cell lysate: Resuspend cells at 1-2 x 106 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or titrated as necessary. • Plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Notes: • All samples should be assayed immediately or stored at -80 °C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples. • A serial dilution will be necessary depending on the total H2O2 or peroxidase present. Extremely high levels of H2O2 (≥ 500 µM final concentration) or peroxidase (≥ 100 mU/mL) can lower the fluorescence because excess H2O2 or peroxidase can further oxidize the reaction product, Resorufin, to nonfluorescent product Resazurin. • Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µ M will oxidize the ADHP probe and could result in erroneous readings. To minimize this

Application Details

	interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2). 4 • Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presense of thiols (above 10 µM).
Assay Procedure:	I. Hydrogen Peroxide 1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate. 2. Add 50 µL of each sample (H2O2 standard, control or unknown) into an individual microtiter plate well. 3. Add 50 µL of ADHP/HRP Working Solution to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the kinetics of the reactions. 4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range. 5. Calculate the concentration of peroxide within samples by comparing the sample RFU to the standard curve. Subtract the value from the zero H2O2 control. II. Peroxidase 1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate. 2. Add 50 µL of each sample (HRP standard, control or unknown) into an individual microtiter plate well. 3. Add 50 µL of ADHP/ H2O2 Working Solution to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the kinetics of the reactions. 4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range. 5. Calculate the concentration of peroxidase within samples by comparing the sample RFU to the standard curve. Subtract the value from the zero HRP control. 6
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the ADHP probe and HRP at -20°C. Avoid multiple freeze/thaw cycles. Store the remaining kit components at 4°C. ADHP is light sensitive, must be stored accordingly. 3

Publications

Product cited in:

Endesfelder, Weichelt, Strauß, Schlör, Sifringer, Scheuer, Bührer, Schmitz: "Neuroprotection by Caffeine in Hyperoxia-Induced Neonatal Brain Injury." in: **International journal of molecular sciences**, Vol. 18, Issue 1, (2017) (PubMed).

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Begieneman, Ter Horst, Rijvers, Meinster, Leen, Pankras, Fritz, Kubat, Musters, van Kuilenburg, Stap, Niessen, Krijnen: "Dopamine induces lipid accumulation, NADPH oxidase-related oxidative stress, and a proinflammatory status of the plasma membrane in H9c2 cells." in: **American journal of physiology. Heart and circulatory physiology**, Vol. 311, Issue 5, pp. H1097-H1107, (2016) (PubMed).

Son, Kim, Nah, Park, Lee, Han, Venkatareddy, Gann, Rodriguez, Burt, Ham, Jung, Hong: "Novel synthetic (E)-2-methoxy-4-(3-(4-methoxyphenyl) prop-1-en-1-yl) phenol inhibits arthritis by targeting signal transducer and activator of transcription 3." in: **Scientific reports**, Vol. 6, pp. 36852, (2016) (PubMed).

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