Datasheet for ABIN2344999

OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)

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Overview

Quantity:	96 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The OxiSelect™ Intracellular ROS Assay Kit is a cell-based assay for measuring antioxidant or ROS activity.
Brand:	OxiSelect™
Sample Type:	Cell Samples
Detection Method:	Fluorometric
Sensitivity:	10 pM
Characteristics:	OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence) is a cell-based assay for measuring hydroxyl, peroxyl, or other reactive oxygen species activity within a cell. The assay employs the cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescin diacetate (DCFH-DA). In brief, DCFH-DA is diffused into cells and is deacetylated by cellular esterases to non-fluorescent 2', 7'-Dichlorodihydrofluorescin (DCFH), which is rapidly oxidized to highly fluorescent 2', 7'- Dichlorodihydrofluorescein (DCF) by ROS (Figure 1). The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The effect of antioxidant or free radical compounds on DCF- DA can be measured against the fluorescence of the provided DCF standard. The kit has a DCF detection sensitivity limit of 10 pM. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Product Details

Components:	1. 20X DCFH-DA : One 500 µL amber tube of a 20 mM solution in methanol. 2. DCF Standard : One 100 µL amber tube of a 1 mM solution in DMSO. 3. Hydrogen Peroxide : One 100 µL amber tube of an 8.821 M solution. 4. 2X Cell Lysis Buffer : One 20 mL bottle. 3
Material not included:	1. Sterile DPBS for washes and buffer dilutions 2. Hank's Balanced Salt Solution (HBSS) 3. Cell culture medium (ie: DMEM +/-10 % FBS) 4. 96-well black or fluorescence microtiter plate 5. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

Target Details

Background:	Accumulation of reactive oxygen species (ROS) coupled with an increase in oxidative stress has been implicated in the pathogenesis of several disease states. The role of oxidative stress in vascular diseases, diabetes, renal ischemia, atherosclerosis, pulmonary pathological states, inflammatory diseases, and cancer has been well established. Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. Measuring the effect of antioxidant therapies and ROS activity intracellularly is crucial to suppressing or treating oxidative stress inducers.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quick ~1 hour protocol • Highly sensitive to 10 pM • Detects the presence of various ROS species
Assay Time:	1 h
Protocol:	Cells are cultured in a 96-well cell culture plate and then pre-incubated with DCFH-DA, which is cell-permeable . The unknown antioxidant or ROS samples are then added to the cells. After a brief incubation, the cells can be read on a standard fluorescence plate reader. The ROS or antioxidant content in unknown samples is determined by comparison with the predetermined DCF standard curve.
Reagent Preparation:	• 1X DCFH-DA: Dilute the 20X DCFH-DA stock solution to 1X in cell culture media, preferably

Application Details

	without FBS. Stir or vortex to homogeneity. Prepare only enough for immediate applications. Notes: • 1X DCFH-DA/media solution contains 5 % methanol. For cells that are sensitive to methanol, we recommend instead preparing a 0.1X (100 µM) solution of DCFH-DA in cell culture media. • Due to light-induced auto-oxidation, DCFH-DA solutions at any concentration must be protected from light. • Hydrogen Peroxide (H2O2): Prepare H2O2 dilutions in DMEM or DPBS as necessary. Do not store diluted solutions. Hydrogen Peroxide may be used as a positive control in the assay, or as a cell treatment.
Assay Procedure:	I. DCF Dye Loading 1. Prepare and mix all reagents thoroughly before use. Each unknown sample should be assayed in duplicate or triplicate. 2. Culture cells in either a clear or black 96-well cell culture plate. Note: If using a black plate, choose an appropriate plate based on your fluorometer's reader (i.e. choose a clear bottom black plate for bottom readers). 3. Remove media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard. 4. Add 100 µL of 1X DCFH-DA/media solution to cells. Incubate at 37 °C for 30-60 minutes. 5. Remove solution. Repeat step three using multiple washes with DPBS or HBSS. Remove the last wash and discard. 6. Treat DCFH-DA loaded cells with desired oxidant or antioxidant in 100 µL medium. II. Quantitation of Fluorescence • Fluorescence microscopy or Flow cytometry: Fluorescence can be analyzed on an inverted fluorescence microscope or by flow cytometry using excitation and emission wavelengths of 480 nm and 530 nm, respectively. • Fluorescence Plate Reader: • Assays performed in black cell culture fluorometric plates: Plate may be read immediately for kinetic analysis or after 1 hour for static analysis. Plates read for kinetic analysis may be read in increments of 1 and 5 minutes up to 1 hour or more as necessary. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm. • Assays performed in clear cell culture plates: After treatment with desired oxidant or antioxidant, carefully remove treatment media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard. Add 100 µL of medium to each well. Add 100 µL of the 2X Cell Lysis Buffer, mix thoroughly and incubate 5 minutes. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C

Handling

Storage Comment:	Upon receipt, store the DCFH-DA and DCF Standard at -20°C. Avoid multiple freeze/thaw cycles. Store the Cell Lysis Buffer and Hydrogen Peroxide at 4°C.

Publications

Product cited in:

Acharya, Nemade, Papadopoulos, Hescheler, Neumaier, Schneider, Rajendra Prasad, Khan, Hemmersbach, Gusmao, Mizi, Papantonis, Sachinidis: "Microgravity-induced stress mechanisms in human stem cell-derived cardiomyocytes." in: **iScience**, Vol. 25, Issue 7, pp. 104577, (2022) (PubMed).

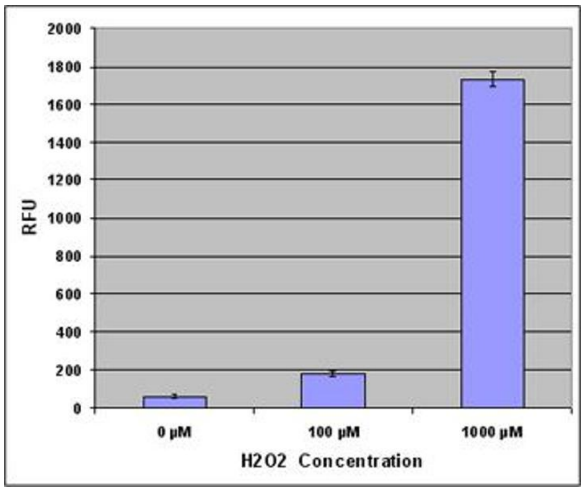
Yao, Zou, Wang, Ji, Yang: "Pinoresinol Diglucoside Alleviates oxLDL-Induced Dysfunction in Human Umbilical Vein Endothelial Cells." in: **Evidence-based complementary and alternative medicine : eCAM**, Vol. 2016, pp. 3124519, (2017) (PubMed).

Golestaneh, Chu, Xiao, Stoleru, Theos: "Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration." in: **Cell death & disease**, Vol. 8, Issue 1, pp. e2537, (2017) (PubMed).

Crookenden, Walker, Heiser, Murray, Dukkipati, Kay, Meier, Moyes, Mitchell, Loor, Roche: "Effects of precalving body condition and prepartum feeding level on gene expression in circulating neutrophils." in: **Journal of dairy science**, Vol. 100, Issue 3, pp. 2310-2322, (2017) (PubMed).

Zhang, Zhao, Sun, Li, Wei, Ashman, Hu: "Different virulence of candida albicans is attributed to the ability of escape from neutrophil extracellular traps by secretion of DNase." in: **American journal of translational research**, Vol. 9, Issue 1, pp. 50-62, (2017) (PubMed).

There are more publications referencing this product on: Product page



Cellular Assay

Image 1. ROS in HeLa Cells Treated with Hydrogen Peroxide. 50,000 HeLa cells in a 96-well plate were pretreated with 1 mM DCFH-DA for 60 minutes at 37ºC. Cells were then treated with hydrogen peroxide for 20 minutes.