Datasheet for ABIN2345012

OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

2 Images | 97 Publications

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Overview

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

Product Details

Purpose:	The OxiSelect™ In Vitro ROS/RNS Assay Kit is an in vitro assay for measuring total ROS/RNS free radical activity.
Brand:	OxiSelect™
Sample Type:	Cell Lysate, Serum, Plasma, Urine
Detection Method:	Fluorometric
Sensitivity:	10 pM
Characteristics:	The OxiSelect™ In Vitro ROS/RNS Assay Kit is an assay for measuring the total free radical presence of a sample. The assay employs a proprietary quenched fluorogenic probe, dichlorodihydrofluorescin DiOxyQ (DCFH-DiOxyQ), which is a specific ROS/RNS probe that is based on similar chemistry to the popular 2', 7'-dichlorodihydrofluorescein diacetate. The DCFH-DiOxyQ probe is first primed with a quench removal reagent, and subsequently stabilized in the highly reactive DCFH form. In this reactive state, ROS and RNS species can react with DCFH, which is rapidly oxidized to the highly fluorescent 2', 7'-dichlorodihydrofluorescein (DCF) (Figure 1). Fluorescence intensity is proportional to the total ROS/RNS levels within the sample. The DCFH-DiOxyQ probe can react with hydrogen peroxide (H O), peroxyl radical (ROO•), nitric oxide (NO), and peroxynitrite anion (ONOO-2 2). These free radical molecules are representative of both ROS and RNS, thus allowing for measurement of the total free radical

Product Details

	population within a sample. OxiSelect™ In Vitro ROS/RNS Assay Kit can also be used to evaluate antioxidant's effect on free radicals. The kit has a detection sensitivity limit of 10 pM for DCF and 40 nM for H2O2 respectively. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.
Components:	1. Priming Reagent : One 250 µL tube of solution. 2. Stabilization Solution (10X) : One 1.5 mL tube of solution. 3. Catalyst (250X) : One 20 µL tube of solution. 4. DCF-DiOxyQ : One 50 µL amber tube of solution in methanol. 5. DCF Standard : One 100 µL amber tube of a 1 mM solution in DMSO. 6. Hydrogen Peroxide : One 100 µL amber tube of an 8.821 M solution.
Material not included:	1. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 2. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 3. Multichannel micropipette reservoir 4. Phosphate Buffered Saline for sample preparations and dilutions 5. 96-well black or fluorescence microtiter plate 6. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

Target Details

Background:	Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well-established molecules responsible for the deleterious effects of oxidative stress. Accumulation of free radicals 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, cancer, as well as ageing 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/RNS activity is crucial to suppressing or treating oxidative stress inducers.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Measures total reactive oxygen species and reactive nitrogen species, including hydrogen peroxide, nitric oxide, peroxyl radical, and peroxynitrite anion • Suitable for use with serum, plasma, urine, cell lysates or cell culture supernatants • Detection sensitivity limit of 10 pM for DCF and 40 nM for hydrogen peroxide

Application Details

Protocol:	Unknown ROS or RNS samples or standards are added to the wells with a catalyst that helps accelerate the oxidative reaction. After a brief incubation, the prepared DCFH probe is added to all wells and the oxidation reaction is allowed to proceed . Samples are measured fluorometrically against a hydrogen peroxide or DCF standard. The assay is performed in a 96-well fluorescence plate format that can be read on a standard fluorescence plate reader. The free radical content in unknown samples is determined by comparison with the predetermined DCF or hydrogen peroxide standard curve.
Reagent Preparation:	• 1X Stabilization Solution: Dilute the 10X Stabilization Solution 1:10 by adding 1.5 mL of solution to 13.5 mL of deionized water. Stir or vortex to homogeneity. Store the solution at 4 °C. • 1X Catalyst: Prior to use, dilute the 250X Catalyst 1:250 in PBS. Vortex thoroughly. Prepare only enough for immediate applications (eg. add 10 µL of Catalyst to 2.49 mL PBS for 50 wells). • DCFH Solution: Prepare only enough DCFH Solution for immediate applications in an amber tube or aluminum foil covered tube. Prepare DCFH Solution by diluting the stock solution of DCF- DiOxyQ 1:5 with Priming Reagent (eg. for 50 assays, add 25 µL DCF-DiOxyQ to 100 µL Priming Reagent). Vortex to homogeneity. Incubate the solution for 30 minutes at room temperature. Next, dilute the reaction 1:40 with 1X Stabilization Solution (eg. for 50 assays, add 125 µL DCF- DiOxyQ/ Priming Reagent reaction to 4.875 mL of Stabilization Solution). Vortex to homogeneity. Protect the solution from light. This solution is now stable in the DCFH form and ready to use. The solution may be stored at -20 °C for up to one week when protected from light. Note: Due to light-induced auto-oxidation, the stock DCF-DiOxyQ solution and all subsequent DCF-DiOxy and DCFH solutions must be protected from light. 4
Sample Preparation:	All samples should be assayed immediately or stored at -80 °C for up to 1-2 months. The assay may be used on cell or tissue lysates, cell culture supernatants, serum, plasma, urine, and other biological fluids. Always run a standard curve with samples. Use PBS for dilution and preparation of samples. Some common detergents and denaturants have been tested for compatibility in the assay (below table). Dilution of samples, and interfering substances, may be necessary for assay compatibility. Substance Compatible Concentration Triton X-100 <1 % NP-40 <1 % SDS <0.1 % Deoxycholate <1 % Tween-20 <0.1 % EDTA <10 mM EGTA <10 mM Glycerol <10 % Table 1. Substance Compatibility Table • Cells or Tissues: Resuspend cells at 1-2 x 107 cells/mL or tissues at 10-50 mg/mL in PBS. Homogenize or sonicate on ice. To remove insoluble particles, spin at 10,000 g for 5 min. The homogenate can be assayed directly or stored at -80 °C as necessary. • Serum, Plasma, Urine or Cell Culture Supernatants: To remove insoluble particles, spin at 10,000 g for 5 min. The supernatant can be assayed directly or stored at -80 °C as necessary.

Application Details

Assay Procedure:	1. Prepare and mix all reagents thoroughly before use. Each sample, including unknown(s) and standard(s), should be assayed in duplicate or triplicate. 2. Add 50 µL of unknown sample or hydrogen peroxide standard to wells of a 96-well plate suitable for fluorescence measurement. 3. Add 50 µL of Catalyst to each well. Mix well and incubate 5 minutes at room temperature. 4. Add 100 µL of DCFH solution to each well. Cover the plate reaction wells to protect them from light and incubate at room temperature for 15-45 minutes. 5. Read the fluorescence with a fluorescence plate reader at 480 nm excitation / 530 nm emission. 6
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C
Storage Comment:	Upon receipt, store the DCF-DiOxyQ and DCF Standard at -20°C. Avoid multiple freeze/thaw cycles. Store all other components at 4°C.

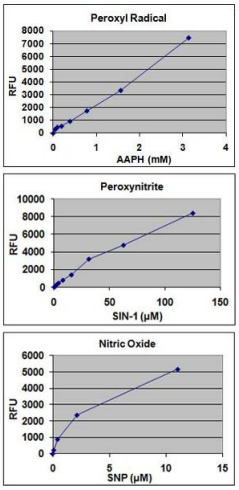
Publications

Product cited in:	Liu, Tang, Li: "Effect and Mechanism Study of Sodium Houttuyfonate on Ventilator-Induced Lung Injury by Inhibiting ROS and Inflammation." in: **Yonsei medical journal**, Vol. 62, Issue 6, pp. 545-554, (2021) (PubMed). Soni, Gandhi, Tarale, Bafana, Pandey, Sivanesan: "Oxidative Stress and Genotoxicity of Zinc Oxide Nanoparticles to Pseudomonas Species, Human Promyelocytic Leukemic (HL-60), and Blood Cells." in: **Biological trace element research**, (2017) (PubMed). Tsai, Su, Chan, Chan: "Nitrosative Stress-Induced Disruption of Baroreflex Neural Circuits in a Rat Model of Hepatic Encephalopathy: A DTI Study." in: **Scientific reports**, Vol. 7, pp. 40111, (2017) (PubMed). Cho, Lee, Song: "Neuronal Cell Death and Degeneration through Increased Nitroxidative Stress and Tau Phosphorylation in HIV-1 Transgenic Rats." in: **PLoS ONE**, Vol. 12, Issue 1, pp. e0169945, (2017) (PubMed). Roche, Heiser, Mitchell, Crookenden, Walker, Kay, Riboni, Loor, Meier: "Strategies to gain body

condition score in pasture-based dairy cows during late lactation and the far-off nonlactating period and their interaction with close-up dry matter intake." in: **Journal of dairy science**, Vol. 100, Issue 3, pp. 1720-1738, (2017) (PubMed).

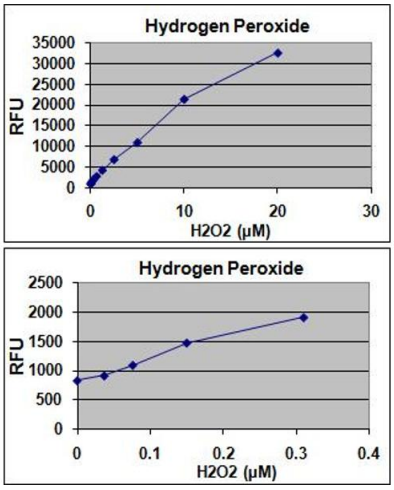
There are more publications referencing this product on: Product page

Images



Activity Assay

Image 1. Detection of Various Free Radical Species. DCF fluorescence curves for AAPH (peroxyl radical generator), SIN-1 (peroxynitrite generator), and SNP (nitric oxide generator).



Activity Assay

Image 2. Hydrogen Peroxide Standard Curve.