Overview

Product name: DCFDA / H2DCFDA - Cellular ROS Assay Kit
Detection method: Fluorescent
Sample type: Adherent cells, Suspension cells
Assay type: Cell-based (quantitative)
Assay time: 0h 40m

Product overview

DCFDA - Cellular ROS Assay Kit / Reactive Oxygen Species Assay Kit (ab113851) uses the cell permeant reagent 2',7' –dichlorofluorescin diacetate (DCFDA, also known as H2DCFDA and as DCFH-DA), a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell.

After diffusion into the cell, DCFDA / H2DCFDA / DCFH-DA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2', 7' –dichlorofluorescein (DCF). DCF is a highly fluorescent compound which can be detected by fluorescence spectroscopy with excitation / emission at 495 nm / 529 nm.

H2DCFDA / DCFH-DA / DCFDA assay protocol / ROS assay protocol summary (microplate):
- collect suspension cells in tube / seed and allow attachment of adherent cells in 96-well plate
- wash in buffer
- stain with DCFDA for 30 min (suspension) / 45 min (adherent), wash with buffer
- if suspension cells, transfer to microplate
- analyze with microplate reader

H2DCFDA / DCFH-DA / DCFDA assay protocol / ROS assay protocol summary (flow cytometry):
- collect cells in tubes
- stain with DCFDA for 30 min (without washing)
- analyze with flow cytometer

H2DCFDA / DCFH-DA / DCFDA assay protocol / ROS assay protocol summary (fluorescent microscopy):
- wash adherent cells with buffer
- stain with DCFDA for 45 min
- wash in buffer
- analyze with fluorescent microscope
- maintain low light conditions to reduce photo-bleaching
Notes
Previously called DCFDA / H2DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit. This kit contains sufficient materials for approximately 300 measurements in microplate format and 70 measurements (35 mL) by flow cytometry. The number of measurements in microscopy is dependent on experimental setup.

This kit is not compatible with fixed samples. Stained cells must be measured live.

Related products
Review the oxidative stress marker and assay guide, or the full metabolism assay guide to learn about more assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also how to assay metabolic function in live cells using your plate reader.

Platform
Microplate reader, Fluor. microscope, Flow cyt.

Properties

| Storage instructions | Store at -20°C. Please refer to protocols. |

<table>
<thead>
<tr>
<th>Components</th>
<th>300 tests</th>
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</thead>
<tbody>
<tr>
<td>Dilution Buffer (10X, sterile)</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Label (20 mM DCFDA, 1000X)</td>
<td>1 x 35μl</td>
</tr>
<tr>
<td>TBHP (55 mM)</td>
<td>1 x 50μl</td>
</tr>
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Images

Effect of anethole on excessive ROS generation in hMSCs. hMSCs were exposed at 2 mM H2O2 for 30 min and incubated for 2 days in presence or absence of 50 μM anethole. ROS was measured by staining the cells with DCFDA cellular ROS detection assay kit according to the manufacturer’s instructions. ROS generation was observed under a fluorescence microscope at 200× magnification.

Image from Rhee YH et al., BMC Cell Biol, 2018, 19: 12, Fig. 3B.; doi: 10.1186/s12860-018-0163-2 Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/
Kobashigawa et al. (Pubmed 25127116) used the DCFDA ROS assay ab113851 to investigate the causes of the protective effects of metformin (Met) treatment in Doxorubicin (Dox) induced cardiotoxicity.

They identified that in metformin treated H9c2 rat immortalized cardiomyoblasts, Met treatment reduced ROS levels induced by Dox (A). Values represent mean ± S.D. (n=4).

In combination with other assays, they developed the hypothesis that Dox induces increased ROS expression, leading to increased calcium levels and cell death, and that Met reduces this effect by increasing AMPK expression.

ab113851 (DCFDA) labeled and unlabeled Jurkat cells were treated with 50 µM tert-butyl Hydrogen Peroxide (tbHP), then analyzed by flow cytometry.
p38 MAPK pathway involved in oxidative injury to HCECs challenged with \textit{C. albicans}. Increased ROS generation in HCECs challenged with \textit{C. albicans} and inhibition by the p38 activation inhibitor SB203580.

Jurkat cells were labeled with DCFDA (20 µM) or unlabeled (none) and then cultured an additional 3 hours with or without 50 µM tert-butyl hydrogen peroxide (TBHP) according to the protocol. Cells were then analyzed on a fluorescent plate reader. Mean +/- standard deviation is plotted for 4 replicates from each condition. TBHP mimics ROS activity to oxidize DCFDA to fluorescent DCF.

Labeled HL60 cells were treated with idarubicin or doxorubicin for 4 hours at multiple doses according to the protocol. At the end of the treatment cells were read end point in a fluorescent plate reader (Perking Elmer-Wallac 1420 Victor 2 Multilabel plate reader). Mean +/- standard deviation is plotted for 3 replicates from each condition. The dotted line represents the mean of 24 replicates of HL60 cells treated with 0.5% DMSO.
Reactive oxygen species (ROS) measured using the DCFDA assay in human primary articular chondrocytes. Cells were treated with 100 µM tert-butyl-hydroperoxide (tBHP) alone (4 h) ± pre-treatment with apigenin.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"