**Product name**
DCFDA / H2DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit

**Detection method**
Fluorescent

**Assay type**
Cell-based (quantitative)

**Product overview**
DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit (ab113851) uses the cell permeant reagent 2',7' –dichlorofluorescin diacetate (DCFDA, also known as H2DCFDA), a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. After diffusion into the cell, DCFDA / H2DCFDA 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 maximum excitation and emission spectra of 495 nm and 529 nm respectively.

This kit contains sufficient materials for approximately 300 measurements in microplate format and 70 measurements (35 mL) by flow cytometry.

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

Store all components at 4°C in the dark. The kits are stable for at least 6 months from receipt. For longer term storage, keep at -20°C to -80°C in the dark.

**Tested applications**
Suitable for: Flow Cyt, Other

**Platform**
Reagents

**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>
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</table>
DCFDA ROS assay used to study Doxorubicin cardiotoxicity
Kobashigawa et al. (Pubmed 28056084)

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.

Applications

Our Abpromise guarantee covers the use of ab113851 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td>Use at an assay dependent concentration.</td>
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</tr>
<tr>
<td>Other</td>
<td>Use at an assay dependent concentration. ROS can also be measured using a fluorescent plate reader.</td>
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Flow Cytometry - DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit (ab113851)
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.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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