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DATA SHEET

Version: 03
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1. Identification

Product name

ROS Detection Assay Kit (DCFDA / H2DCFDA)

5 x 96 assays

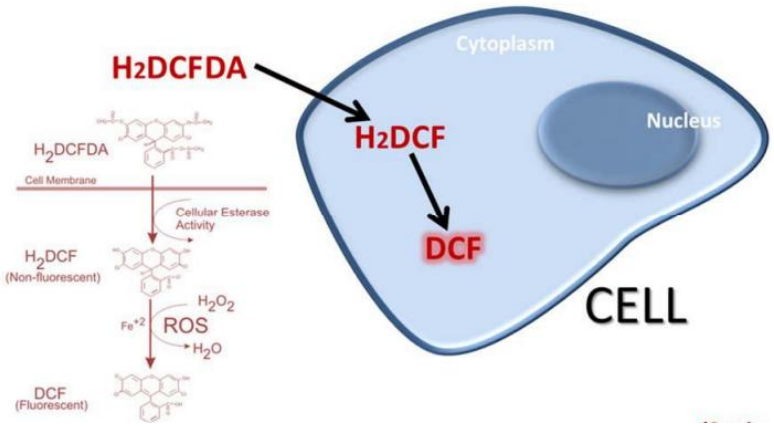
Cat. No: **CA093**

2. Description

Reactive oxygen species (ROS) include a number of reactive molecules and free radicals, derived from molecular oxygen, that damage DNA and DNA and oxidize proteins and lipids (lipid peroxidation). The most common ROS include superoxide anion (O2 •-), Hidrogen peroxide (H2O2), hydroxyl radical (HO•) and singlet oxygen (1O2), all of which are more reactive than oxygen (O2) itself. The molecules are produced during the electron transport of mitochondrial aerobic respiration or by oxidoreductase enzymes and metal catalysed oxidation.

ROS Detection Assay kit uses the cell permeant reagent Dichlorodihydrofluorescein-diacetate (H2DCFDA), a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell.

After diffusion into the cell, the acetyl groups on H2DCF-DA are cleaved by intracellular esterase to yield the non-fluorescent compound which is rapidly oxidized to highly fluorescent, 2',7'-Dichlorodihydrofluorescein by ROS. The fluorescence intensity isproportional to the ROS levels within the cell cytosol



3. Composition

Item	Quantity
Fluorescent Substrate (H_2DCFDA)	5 mg
10X Assay buffer	50 mL
Positive Control: H2O2 (9.7M)	500 µL
DMSO	1 mL

4. Storage

Store the kit at 2-8°C.

5. DFC-DA stock solution preparation

H2DCFDA is supplied lyophilized and should be reconstituted in 500µl DMSO to yield a 20mM stock solution.
Upon reconstitution, the stock solution should be stored at -20°C to -80°C in the dark. Gently mix before use. Avoid multiple freeze/thaw cycles.

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7. Hydrogen Peroxide preparation

Prepare H_2O_2 dilutions in 1X Assay Buffer. Do not store diluted solutions. Hydrogen Peroxide may be used as positive control in the assay, or as a cell treatment.

8. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. **Canvax Reagents S.L.U.** shall not be held liable for any damage resulting from handling or from contact with the above product.

Assay procedure

- ✓ Equilibrate all materials and prepared reagents to room temperature prior to use.
- ✓ It is recommended to assay all controls and samples in duplicate or triplicate.
- ✓ It is recommended to use black plates for fluorometric assays.

A-Preparation of working solutions

1. Prepare **1X Assay Buffer** by adding 10 mL **10X Assay Buffer** to 90 mL deionized water. Mix gently and thoroughly.
2. Prepare a **working H_2DCFDA solution** by adding the appropriate volume of 20 mM H_2DCFDA to 1X Assay Buffer. Vortex to evenly disperse the dye.

Vortexing for about 15 to 30 seconds is usually optimal. Excessive vortexing may hasten decomposition of the dye, as it is subject to hydrolysis; on the other hand, shorter vortexing times may result in incomplete dispersion of the dye.

The exact concentration of DCFDA required will depend on the cell line being used but a general starting range would be 10 - 50 µM. *Exact concentrations have to be determined on an individual basis by the end user. This solution is unstable and must be used immediately for staining cells in culture.*

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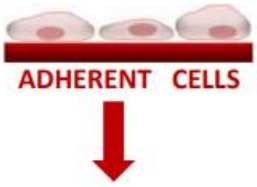
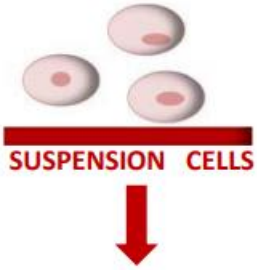
B- Assay





1. Stain cells in 1X buffer containing the probe (**working H_2DCFDA solution**) to provide a final working concentration of ~1–50 µM dye (100 µL per well for a 96-well format).
   The optimal working concentration for your application must be empirically determined.
2. Incubate at 37ºC for 30–60 minutes.
3. Wash cells once in **1X Assay Buffer**.

Add 100ul/well of treatment →

4. Treat the cells with the test ROS inducing agents or H_2O_2 (Positive control: concentrations between 25 -100 µM).
5. Incubate for desired period of time. *Proper incubation time may need to be determined experimentally.*
6. The intensity of the signal can be easily measured using a fluorescence microplate reader at Ex/Em = 485/530 nm, or a fluorescent microscope with TRITC filter or a flow-cytometry in FL1 channel.