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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

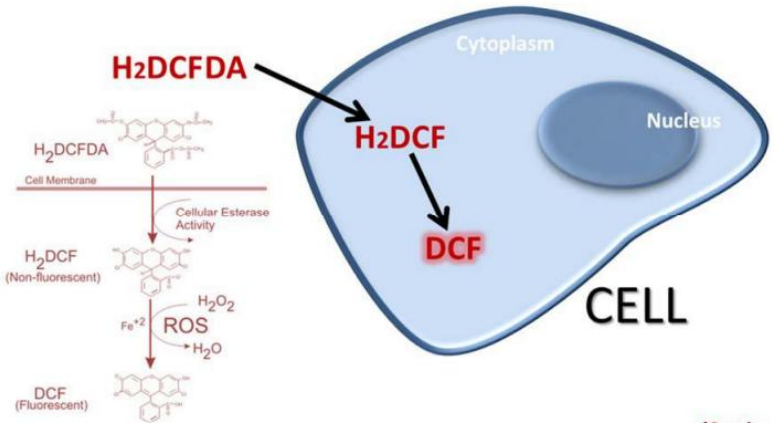
Cat. No: **CA093**

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

A- Preparation of working solutions

1. Prepare 1X Assay Buffer

Prepare **1X Assay Buffer** by adding **10 mL 10X Assay Buffer** to **90 mL deionized water**. Mix gently and thoroughly.

2. Prepare H_2DCFDA working solution

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.*

3. Prepare H_2O_2 solutions

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

H_2DCFDA loading and subsequent manipulations should be performed protected from light whenever possible.

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B- Assay

B1. Adherent cells

1. Cell preparation

Remove the culture medium and wash the cells once with **1X Assay Buffer**.

2. H_2DCFDA loading

Add **100 µL/well** of **1X Assay Buffer containing H_2DCFDA working solution** to provide a final working concentration of approximately **1–50 µM dye**.

The exact concentration of H_2DCFDA required will depend on the cell line being used and must be determined empirically.

3. Incubation

Incubate at **37°C for 45-60 minutes**, protected from light.

4. Probe washing

Wash cells once with **1X Assay Buffer** to remove excess extracellular probe.

5. Treatment

Add **100 µL/well** of treatment solution prepared in **1X Assay Buffer**.

Treat the cells with the desired ROS-inducing agent or with **H_2O_2 (25–100 µM)** as a positive control.

6. Treatment incubation

Incubate for the desired period of time.

The optimal incubation time depends on the cell type and treatment used and may need to be determined experimentally.

Important Note: Do not perform any washing step after treatment with H_2O_2 or any other ROS-inducing stimulus. Measure fluorescence directly after the treatment incubation period. Intracellular ROS generation is a dynamic process, and additional washing steps may affect the detectable fluorescence signal.

7. Fluorescence measurement

Measure fluorescence immediately after treatment incubation using:

- a fluorescence microplate reader at Ex/Em = 485/530 nm, or
- a fluorescence microscope with a FITC filter, or
- flow cytometry in the FL1 channel.

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B2. Suspension cells

1. H_2DCFDA loading

Resuspend cells in **1X Assay Buffer containing H_2DCFDA working solution** to provide a final working concentration of approximately **1–50 µM dye**.

The exact concentration of H_2DCFDA required will depend on the cell line being used and must be determined empirically.

2. Incubation

Incubate at **37°C for 30–60 minutes**, protected from light.

3. Probe washing

Centrifuge the cells, remove the supernatant, and resuspend the pellet in fresh **1X Assay Buffer** to remove excess extracellular probe.

4. Treatment

Add the desired ROS-inducing agent or **H_2O_2 (25–100 µM)** prepared in **1X Assay Buffer**.

5. Treatment incubation

Incubate for the desired period of time.

The optimal incubation time depends on the cell type and treatment used and may need to be determined experimentally.

Important Note: Do not perform any washing step after treatment with H_2O_2 or any other ROS-inducing stimulus. Measure fluorescence directly after the treatment incubation period. Intracellular ROS generation is a dynamic process, and additional washing steps may reduce or alter the detectable fluorescence signal.

6. Fluorescence measurement

Measure fluorescence immediately after treatment incubation using:

- a fluorescence microplate reader at Ex/Em = 485/530 nm, or
- a fluorescence microscope with a FITC filter, or
- flow cytometry in the FL1 channel.