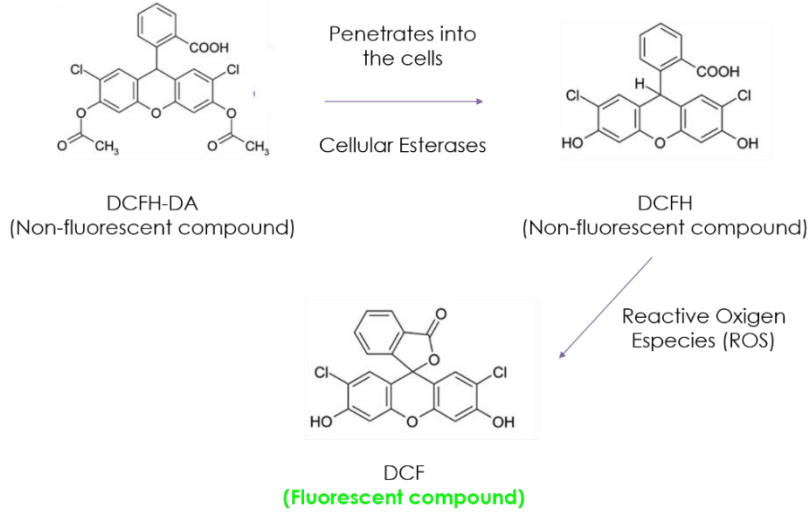
DCFH-DA PROBE | INTRACELLULAR ROS ASSAY

KP06003-250/500/1000 Tests

DESCRIPTION AND USE

Reactive Oxygen Species (ROS) can be induced by some stress conditions like exposure to oxidant or drugs. This fact leads to oxidative stress. ROS induce damage in DNA, protein and lipids with important consequences in cells.

Cell permeant reagent 2'-7'dichlorofluorescin diacetate (DCFH-DA) is a fluorogenic dye that measures hydroxyl, peroxyl and other ROS activity. After cell uptake, 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 fluorescent compound which can be detected by fluorimeter, flowcytometer or fluorescence microscope with a maximum excitation and emission spectra of 495 nm and 529 nm respectively.



Scheme 1. Principle of the assay reaction.

DCFH-DA probe is a READY TO USE probe suitable for: Flow cytometer, Microscopy, Fluorimeter

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MATERIALS SUPPLIED

Item	No. Tests	Quantity
Reagent A	250	1
	500	2
	1000	4
Reagent B	250	1
	500	2
	1000	4
Reagent C	250	1
	500	1
	1000	1

STORAGE AND STABILITY

On receipt store kit components at -20 °C. Do not use after the expiration date stated on the packaging.

REAGENT PREPARATION

Dilution buffer: Prepare 1X dilution buffer by diluting Reagent A (40X) in a 1:40 ratio with ddH_2O. Example: Dilute 0.5 mL of reagent A in 19.5 mL of ddH_2O and mix gently.

- Store the dilution buffer at 4 °C and equilibrate to 37 °C before using it.

DCFH-DA Probe Working Solution: Dilute Reagent B (DCFH-DA Probe, 20 mM) with the desired amount of the Dilution Buffer. The exact concentration of DCFH-DA required will depend on the cell line being used but a general starting range would be 10 – 25 µM. Exact concentrations must be determined on an individual basis by the end user. Before preparing DCFH-DA Probe Working Solution, consider the number of tests to be carried out and therefore the volume of Working Solution required.

Positive control: Dilute Reagent C (55 mM tert-butyl hydroperoxide (TBHP)) to a concentration, in order to obtain a final concentration in the well of ~100 µM (increase or decrease based on the sensitivity and response of the cells). Example: In 96-well plates with 100 µL of medium, add 1 µL of diluted Reagent C (TBHP, 10 mM) 4-6 hours before performing the assay.

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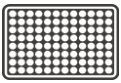
ASSAY PROTOCOL

For 96-well microplate reader

		Adherent cells	Suspension cells
1		Seed adherent cells at 25 x 10^3 per well one day before performing the assay	Grow suspension cells in sufficient amount. *(In the step 5 you will need 100 x 10^3 cells per group)*
2		Remove the media and add 100 µL/well of the **Dilution buffer**	Collect cells and wash by centrifugation in PBS
3		Remove Dilution buffer and add 100 µL/well of the **Probe Working Solution**	Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of the **Probe Working Solution**
4		Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough	Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough
5		Remove media and add at least 100 µL of PBS. Measure fluorescence **(λ_{exc}: 485 nm/ λ_{em}: 535 nm)** immediately	Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate with 100000 stained cells/well and measure fluorescence **(λ_{exc}: 485 nm/ λ_{em}: 535 nm)** immediately

*X represents the volume of DCFH-DA to obtain the optimal concentration related to the cell line used.

NOTE: To create positive controls, oxidative activity is stimulated with Reagent C prior to analysis (See Reagent Preparation).

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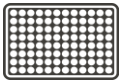
For flow cytometer

1  Grow cells (adherent or suspension) so that on the day of the experiment there are at least 15 x 10^3 cells per assayed condition (treatment, dose, time). Include in the calculation enough cells for control

2  Harvest cells and ensure a single cell suspension by gently pipetting up and down suspension cells or by fully detaching adherent cells (e.g. trypsinize and quench with media).

3  Stain cells in culture media with **10-25 µM DCFH-DA Probe Working Solution** and incubate for 30 minutes at 37 °C. Once the incubation is completed, DO NOT wash the cells

4  After staining, treat the cells with compound(s) of interest and ensure that appropriate controls are included. If using THBP as positive control, optimal signal is obtained after 4 hours of treatment.

5  Analyze on flow cytometer. Establish forward and side scatter gates to exclude debris and cellular aggregates from analysis. DCF should be excited by the 488 nm laser and should be detected at 535 nm.

DATA ANALYSIS

For 96-well microplate reader

Subtract blank readings from all measurements and determine fold change from assay control.

For Flow Cytometer

Exclude debris and isolate cell population of interest with gating. Using mean fluorescent intensity, determine fold change between control and treated samples.

RELATED PRODUCTS

Product	Reference
Superoxide dismutase (SOD) Activity Assay Kit	KB03011
Xanthine Oxidase Activity Assay Kit	KB03032
ABTS Assay Kit	KF01002

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