DHE PROBE | INTRACELLULAR ROS ASSAY

KP06002-250/500/1000 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 Dihydroethidium (DHE) is a fluorogenic dye that is useful for the detection of reactive oxygen species. DHE has been shown to be oxidized by superoxide to form 2-hydroxyethidium (2-OH-E^+) ($\lambda_{excitation}$: 500-530 nm/ $\lambda_{emission}$: 590-620 nm) or by non-specific oxidation by other sources of reactive oxygen species to form ethidium (E^+) ($\lambda_{excitation}$: 480 nm/ $\lambda_{emission}$: 576 nm).

DHE probe is a READY TO USE probe suitable for: Flow cytometer, Microscopy, Fluorimeter

MATERIALS SUPPLIED

Item	No. Tests	Quantity
DHE Probe	250	1
	500	2
	1000	4

STORAGE AND STABILITY

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

REAGENT PREPARATION

DHE Probe Working Solution: Dilute Reagent A (DHE Probe, 5 mM,1000X) in a 1:1000 ratio with PBS (not included). Use the required amount of DHE and PBS for your tests. Example: 1 µL of DHE probe (1000X) with 999 µL of PBS.

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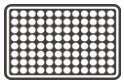
ASSAY PROTOCOL

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 PBS	Collect cells and wash by centrifugation in PBS
3		Remove PBS and stain cells by adding 100 µL/well of **DHE Probe Working Solution**	Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of **DHE 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}: 510 nm/ λ_{em}: 600 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}: 510 nm/ λ_{em}: 600 nm)** immediately

For flow cytometer: Follow the protocol for suspension cells, avoiding step 5.

RELATED PRODUCTS

Product	Reference
NAD/NADH Quantification Assay Kit	KB03033
Xanthine Oxidase Activity Assay Kit	KB03032
ORAC Assay Kit	KF01004

FOR RESEARCH USE ONLY

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