DHR 123 PROBE | INTRACELLULAR ROS ASSAY

KP06004-250/500/1000 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 Dihydrorhodamine 123 (DHR 123) is a fluorogenic dye that is useful for the detection of reactive oxygen species such as peroxide and peroxynitrite. After cell uptake, DHR 123 is oxidized by ROS into a fluorescent compound. It seems that neither the superoxide, the NO, nor the hydrogen peroxide by themselves, are capable of oxidizing DHR. These ROS, are thought to combine with other cellular components such as cytochrome c oxidase or Fe^{2+} in order to oxidize DHR 123 to its fluorescent derivative Rhodamine 123.

Rhodamine 123 can be detected by fluorimeter, flow cytometer or fluorescence microscope with a maximum excitation and emission spectra of 500 and 536 nm, respectively. It can be also detected by absorbance spectroscopy at 500 nm (ε = 78,800 $M^{-1}cm^{-1}$).

DHR 123 probe is a READY TO USE probe suitable for: Flow cytometer, Microscopy, Fluorimeter

MATERIALS SUPPLIED

Item	No. Tests	Quantity
DHR 123 Probe	250	1
	500	2
	1000	4

STORAGE AND STABILITY

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

REAGENT PREPARATION

DHR 123 Probe Working Solution : Dilute Reagent A (DHR 123 Probe, 5 mM,1000X) in a 1:1000 ratio with PBS (not included). Use the required amount of DHR 123 and PBS for your tests. Example: 1 µL of DHR 123 probe (1000X) with 999 µL of PBS.

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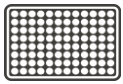
ASSAY PROTOCOL

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 **DHR 123 Probe Working Solution**	Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of **DHR 123 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}: 500 nm/ λ_{em}: 536 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}: 500 nm/ λ_{em}: 536 nm)** immediately

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

RELATED PRODUCTS

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

FOR RESEARCH USE ONLY

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