Multiprobe REDOX | INTRACELLULAR ROS ASSAY

KP06005-250/500/1000 Tests

DESCRIPTION AND USE

Fluorescent probes mix (DHE, DHR 123, and DCFH-DA) is a useful tool for measuring free radicals like Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS). Is for that reason, the Multiprobe REDOX kit contains the most used fluorescent probes, has a great advantage because you can measure different kinds of free radicals with just one product.

- Cell permeant reagent **Dihydroethidium (DHE)** is a fluorogenic dye that is useful for the detection of reactive oxygen species.
- Cell permeant reagent **2'-7'dichlorofluorescin diacetate (DCFH-DA)** is a fluorogenic dye that measures hydroxyl, peroxyl, and other ROS activity.
- 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.

MATERIALS SUPPLIED

Item	No. Tests	Quantity
DHE Probe	250	1
	500	2
	1000	4
DCFH-DA Probe	250	1
	500	2
	1000	4
DHR 123 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.

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REAGENT PREPARATION

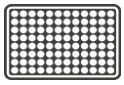
DHE Probe Working Solution: Dilute DHE Probe (5 mM,1000X) in a 1:1000 ratio with PBS (not included). Use the required amount of DHE and PBS for your tests. Example: 1 µL of DHE probe (1000X) with 999 µL of PBS.

DCFH-DA Probe Working Solution: Dilute DCFH-DA Probe (20 mM) with PBS (not included). 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.

DHR 123 Probe Working Solution: Dilute 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.

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 **Probe Working Solution** (DHE, DCFH-DA, or DHR 123)	Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of **Probe Working Solution** (DHE, DCFH-DA, or DHR 123)
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* immediately	Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate with 100000 stained cells/well and measure fluorescence* immediately

DCFH-DA: λ_{exc}: 485 nm/λ_{em}: 535 nm
DHE: λ_{exc}: 510 nm/λ_{em}: 535 nm
DHR 123: λ_{exc}: 500 nm/λ_{em}: 536 nm

FOR RESEARCH USE ONLY

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

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
NAD/NADH Quantification Assay Kit	KB03033
Xanthine Oxidase Activity Assay Kit	KB03032
MDA-TBARS Assay Kit	KB03016

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