DHR 123 Redox Probe

(Cat. # BAQ043, BAQ044, BAQ045)

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INTRODUCTION

Reactive Oxygen Species (ROS) can be induced by stress conditions, such as exposure to oxidants or drugs. These then lead to oxidative stress. ROS induced damage in DNA, protein and lipids can lead to 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 (ROS) 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. TheseROS 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}.

ITEM(S) SUPPLIED

Description	BAQ043 250 tests	BAQ044 500 tests	BAQ045 1000 tests
DHR 123	1 vial	2 vials	4 vials

STORAGE CONDITIONS

The kit is supplied at ambient temperature. Store all the reagents at ambient temperature. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- PBS
- Fluorometer, flow cytometer, fluorescent microscope

PREPARATION BEFORE USE

Dilute DHR 123 probe (1000X) with PBS (not included). Use the required amount of DHR and PBS for your tests. Example: 1 µL of DHR probe (1000X) with 999 µL of PBS.

PROTOCOL FOR ADHERENT CELLS

1. Seed adherent cells at 25 x 10^3 per well one day before performing the assay.
2. Remove the media and add 100 µL/well of PBS.
3. Remove PBS and stain cells by adding 100 µL/well of diluted DHR 123 probe.
4. Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is sufficient.

5. Remove probe and add at least 100 µL of PBS. Measure fluorescence immediately.

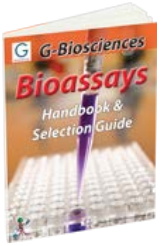
PROTOCOL FOR SUSPENSION CELLS

NOTE: *To create positive controls, oxidative activity is stimulated with reagent C prior to analysis*

1. Grow suspension cells in sufficient amount. (In the step 5 you will need 100 x 10^3 cells per group).
2. Collect and wash cells with PBS using centrifugation.
3. Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of diluted DHR 123 probe.
4. Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.
5. Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate with 100,000 stained cells/well and measure fluorescence immediately.
   NOTE: *For flow cytometry follow the protocol for suspension cells up to point 5.*

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

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