DHE Probe

KP-06-002

250/500/1000 test

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

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- This kit is for R&D use only

Introduction

Reactive Oxygen Species 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 (ROS). DHE has been shown to be oxidized by superoxide to form 2-hydroxyethidium (2-OH-E+) (ex 500-530 nm/em 590-620 nm) or by non-specific oxidation by other sources of reactive oxygen species (ROS) to form ethidium (E+) (ex 480 nm/em 576 nm).

Materials

BQCkit DHE Probe *KP06003-250 tests* contains:

Product	Quantity	Storage
DHE	1 vial	RT

BQCkit DHE Probe *KP06003-500 tests* contains:

Product	Quantity	Storage
DHE	2 vials	RT

BQCkit DHE Probe *KP06003-1000 tests* contains:

Product	Quantity	Storage
DHE	4 vials	RT

Reagent Preparation

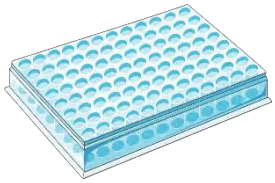
Dilute DHE probe (1000X) with PBS (not included). Use the required amount of DHE and PBS for your tests.

Example: 1 µL of DHE probe (1000X) with 999 µL of PBS.

Assay Protocol

Short protocol:

1



-Adherent cells-

Seed adherent cells at 25 x 10^3 per well one day before performing the assay.

-Suspension cells-

Grow suspension cells in sufficient amount. (In the step 5 you will need 100 x 10^3 cells per group).

2

-Adherent cells-

Remove the media and add 100 µL/well of PBS.

-Suspension cells-

Collect cells and wash by centrifugation in PBS.

3

-Adherent cells-

Remove PBS and stain cells by adding 100 µL/well of previously diluted DHE (see Reagent Preparation).

-Suspension cells-

Resuspend cells at a density of 1x10^6 cells/mL. Stain the cells with the desired volume of previously diluted DHE (see Reagent Preparation).

4		-Adherent cells- Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.	-Suspension cells- Incubate at cells' optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.
5	 Ex/Em= 510/600 nm	-Adherent cells- Remove media and add at least 100 µL of PBS. Measure fluorescence immediately.	-Suspension cells- Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate with 100,000 stained cells/well and measure fluorescence immediately.

FLOW Cytometer: For cytometer application, follow the protocol for suspension cells, avoiding point 5.

Warranties and Limitation of Liability

Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by Bioquochem´s gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer´s exclusive remedy and Bioquochem´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.