# Multiprobe REDOX Assay

## (Cat. # BAQ046, BAQ047, BAQ048)

## 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 2'-7'dichlorofluorescin diacetate (DCFH-DA) is a fluorogenic dye that measures hydroxyl, peroxyl and other ROS activity. After cell uptake, DCFH-DA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2'-7'dichlorofluorescein (DCF). DCF is a fluorescent compound which can be detected by fluorimeter, flow cytometer or fluorescence microscope with a maximum excitation and emission spectra of 495 nm and 529 nm respectively.

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).

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. 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 ($\varepsilon$ = 78,800 $M^{-1}$ $cm^{-1}$).

## ITEM(S) SUPPLIED

| **Description** | **BAQ046 250 tests** | **BAQ047 500 tests** | **BAQ048 1000 tests** |
|---|---|---|---|
| DCFH-DA probe (20mM) | 1 vial | 2 vials | 4 vials |
| DHE probe (5mM) 1000x | 1 vial | 2 vials | 4 vials |
| DHR 123 probe (5mM) 1000x | 1 vial | 2 vials | 4 vials |

## STORAGE CONDITIONS

The kit is supplied on blue ice. Store all the reagents as indicated on the individual labels. 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

***DCFH-DA probe:***

The exact concentration of DCFDA 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.

***DHE probe:***

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.

***DHR 123 probe:***

Dilute DHR 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 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.

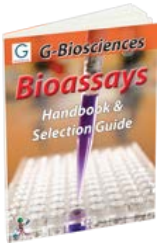
## PROTOCOL FOR SUSPENSION CELLS

***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 1x$10^6$ cells/mL. Stain the cells with the desired volume of diluted 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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