DCFH-DA Redox Probe

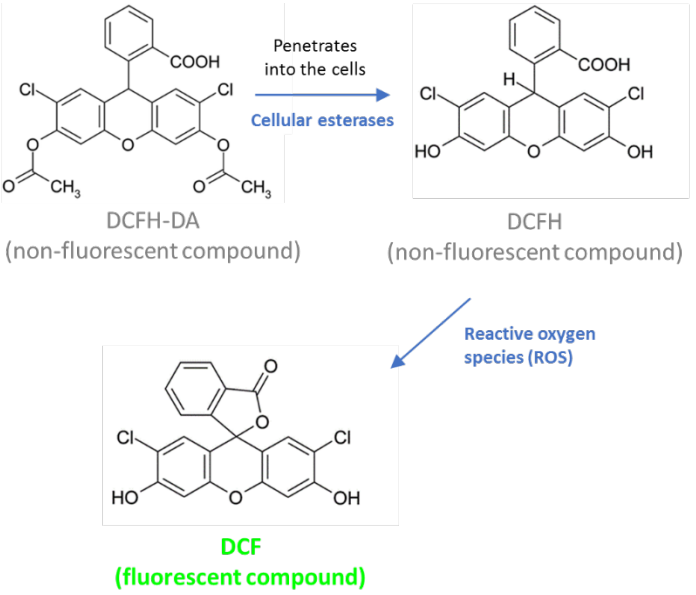
(Cat. # BAQ037, BAQ038, BAQ039)

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 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.

ROS Assay kit, uses 2'-7'dichlorofluorescin diacetate (DCFH-DA), a cell permeant reagent fluorogenic dye that measures hydroxyl, peroxyl and other ROS activity in the cell. 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).



ITEM(S) SUPPLIED

Description	BAQ037 250 tests	BAQ038 500 tests	BAQ039 1000 tests
Reagent A (Dilution Buffer 40X)	1 vial	2 vials	4 vials
Reagent B (Probe (20mM))	1 vial	2 vials	4 vials
Reagent C (Positive control-55mM)	1 vial	1 vial	1 vial

STORAGE CONDITIONS

The kit is supplied on blue ice. Store all the reagents at -20°C. 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

Reagent A (Dilution buffer):

Prepare 1x dilution buffer by diluting Reagent A in ddH_2O.

Example: Dilute 0.5 mL of reagent A in 19.5 mL of double distillated water and mix gently.

Store at 4°C and equilibrate to 37°C before use it.

Reagent B (Probe):

Dilute Reagent B with the desired amount of Reagent A (previously diluted). This will be called Probe Working Solution.

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.

Reagent C (Positive control):

Dilute tert-butyl hydroperoxide (55 mM) to a concentration, in order to obtain a final concentration in the well of ~100 µM (increase or decrease based on the sensitivity and response of the cells).

Example: In 96 well plates with 100 µL of medium, add 1 µL of Reagent C (TBHP, 10 mM) 4-6 hours before performing the assay, in order to create your positive control.

PROTOCOL FOR ADHERENT CELLS

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

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 diluted Reagent A.
3. Remove Reagent A and stain cells by adding 100 µL/well of Probe Working Solution.
4. 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.

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 Probe Working Solution.
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 4.*

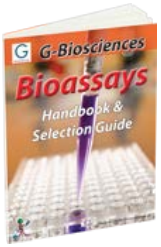
PROTOCOL FORFLOW CYTOMETER

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

1. Grow cells (adherent or suspension) so that on the day of the experiment there are at least 15 x 10^3 cells per assayed condition (treatment, dose, time). Include in the calculation enough cells for a control
2. Harvest cells and ensure a single cell suspension by gently pipetting up and down suspension cells or by fully detaching adherent cells (e.g. trypsinize and quench with media).
3. Stain cells in culture media with 10-25 µM DCFH-DA and incubate for 30 minutes at 37°C. Once the incubation is completed, DO NOT wash the cells
4. After staining, treat the cells with compound(s) of interest and ensure that appropriate controls are included. If using THBP as positive control, optimal signal is obtained after 4 hours of treatment.
5. Analyze on flow cytometer. Establish forward and side scatter gates to exclude debris and cellular aggregates from analysis. DCF should be excited by the 488 nm laser and should be detected at 535 nm.

RELATED PRODUCTS

Download our Bioassays Handbook.



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