DCFH-DA
ROS Assay Kit

KP-06-003

250-500-1000 test

BQCkit

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All chemicals should be handled with care

- 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 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, flowcytometer or fluorescence microscope with a maximum excitation and emission spectra of 495 nm and 529 nm respectively.

Materials

BQCkit DCFH-DA Assay kit *KP06003-250 tests* contains:

Product	Quantity	Storage
Reagent A (Dilution Buffer 40X)	1 vial	-20ºC
Reagent B (Probe (20mM))	1 vial	-20ºC
Reagent C (Positive control-55mM)	1 vial	-20ºC

BQCkit DCFH-DA Assay kit *KP06003-500 tests* contains:

Product	Quantity	Storage
Reagent A (Dilution Buffer 40X)	2 vials	-20ºC
Reagent B (Probe (20mM))	2 vials	-20ºC
Reagent C (Positive control-55mM)	1 vial	-20ºC

BQCkit DCFH-DA Assay kit *KP06003-1000 tests* contains

Product	Quantity	Storage
Reagent A (Dilution Buffer 40X)	4 vials	-20ºC
Reagent B (Probe (20mM))	4 vials	-20ºC
Reagent C (Positive control-55mM)	1 vial	-20ºC

Assay Principle

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).**

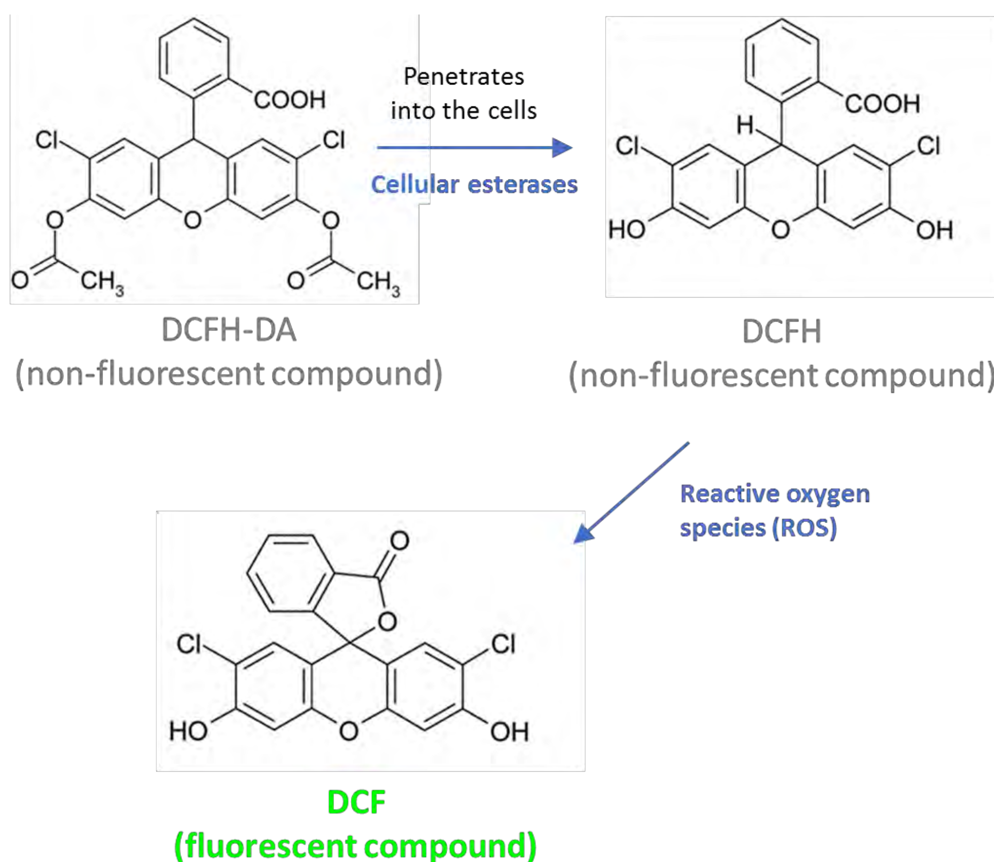


Figure 1. *Principle of the assay reaction*

Reagent Preparation

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).

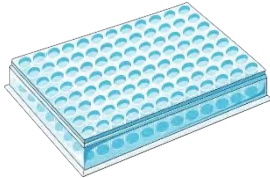
Reagent Preparation

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.

Assay Protocol

Protocol for microplate reader:

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 µ**L/well of previously diluted Reagent A (see Reagent Preparation).

-Suspension cells-

Collect cells and wash by centrifugation in PBS.

3

-Adherent cells-

Remove Reagent A and add **100 µL**/well of the Probe Working Solution previously prepared (see Reagent Preparation).

-Suspension cells-

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

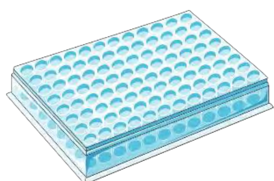
Step		-Adherent cells-	-Suspension cells-
4		Incubate at cell´s optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.	Incubate at cell´s optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.
5	Ex/Em= 485/535 nm	Remove and add at least 100 **µL** of PBS and measure fluorescence immediately.	Wash cells by centrifugation. Resuspend cells in PBS, seed in 96-well microplate with 100,000 stained cells/well and measure fluorescence immediately.

*X represents the volume of DCFH-DA to obtain the optimal concentration related to the cell line used.

NOTE: To create positive controls, oxidative activity is stimulated with reagent C prior to analysis (See Reagent Preparation).

Assay Protocol

Protocol for flow cytometer:

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 control

2

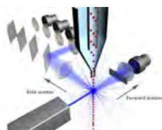
Harvest cells and ensure a single cell suspension by gently pipetting up and down suspension cells or by fully detaching adherent cells (e.g. trypsinize and quench with media).

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.

Data Analysis

Microplate: Subtract blank readings from all measurements and determine fold change from assay control.

Flow cytometry: Exclude debris and isolate cell population of interest with gating. Using mean fluorescent intensity, determine fold change between control and treated samples.

Warranties and Limitation of Liability

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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.