

# Multiprobe Redox Assay Kit

*KP-06-005*

*250/500/1000 test*

# **BOCKit**

*A brand of*  **BioQuoChem**



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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'dichlorofluorescein diacetate** (DCFH-DA) is a fluorogenic dye that measures hydroxyl, peroxy and other ROS activity.

Cell permeant reagent Dihydroethidium (DHE) is a fluorogenic dye that is useful for the detection of reactive oxygen species (ROS).

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.

## Materials

BQCKit Multiprobe Redox Assay kit *KP-06-005 250 tests* contains:

Product	Quantity	Storage
DCFH-DA probe (20mM)	1 vial	-20°C
DHE probe (5mM) 1000x	1 vial	-20°C
DHR 123 probe (5mM) 1000x	1 vial	-20°C

BQCKit Multiredox probe Assay kit *KP-06-005 500 tests* contains:

Product	Quantity	Storage
DCFH-DA probe (20mM)	2 vials	-20°C
DHE probe (5mM) 1000x	2 vials	-20°C
DHR 123 probe (5mM) 1000x	2 vials	-20°C

BQCKit Multiredox probe Assay kit *KP-06-005 1000 tests* contains:

Product	Quantity	Storage
DCFH-DA probe (20mM)	4 vials	-20°C
DHE probe (5mM) 1000x	4 vials	-20°C
DHR 123 probe (5mM) 1000x	4 vials	-20°C

## *Assay Principle*

### *DCFH-DA probe*

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.

### *DHE probe*

DHE has been shown to be oxidized by superoxide to form 2-hydroxyethidium (2-OH-E<sup>+</sup>) (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<sup>+</sup>) (ex 480 nm/em 576 nm).

### *DHR 123 probe*

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<sup>2+</sup> in order to oxidize DHR 123 to its fluorescent derivative Rhodamine 123.

## *Assay Principle*

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

## ***Reagent Preparation***

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  $\mu\text{M}$ .

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  $\mu\text{L}$  of DHE probe (1000X) with 999  $\mu\text{L}$  of PBS.

DHR 123 probe:

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

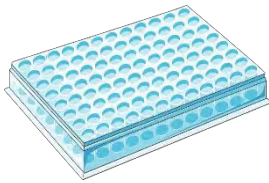
**Example:** 1  $\mu\text{L}$  of DHR probe (1000X) with 999  $\mu\text{L}$  of PBS.



# Assay Protocol

Protocol for microplate reader:

1



-Adherent cells-

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

-Suspension cells-

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

2



-Adherent cells-

Remove the media and add  $100 \mu\text{L}$ /well of Phosphate buffer (PBS).

-Suspension cells-

Collect cells and wash by centrifugation in phosphate buffer (PBS).

3



-Adherent cells-

Remove PBS and **add  $100 \mu\text{L}$** /well of previously diluted probe (DCFH-DA; DHE or DHR 123). (See Reagents Preparation)

-Suspension cells-

Resuspend cells at a density of  $1 \times 10^6$  cells/mL. Stain the cells with the desired volume of previously diluted probe (DCFH-DA; DHE or DHR 123) (see Reagent Preparation).

# Assay Protocol

4



-Adherent cells-

Incubate at cell's optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.

-Suspension cells-

Incubate at cell's optimal temperature in dark conditions. An incubation time of 15–60 minutes is enough.

5



Adherent cells-

Remove and add at least 100  $\mu\text{L}$  of PBS and 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\*.

\*DCFH-DA: ex/em 485 nm/535 nm  
DHE: ex/em 510 nm/600 nm  
DHR 123: ex/em 500 nm/536 nm

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

## *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***

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](http://www.bqckit.com).