ROS Detection Assay Kit

(Fluorometric Readout based on H_2DCFDA dye)

Ordering info

TBK0530. 500 reactions

Description

ROS Detection Assay Kit is a widely used kit designed to detect reactive oxygen species (ROS), highly reactive molecules containing oxygen such as free radicals like superoxide anions ($O_2\bullet^-$), hydroxyl radical ($HO\bullet$) and non-radical compounds like hydrogen peroxide (H_2O_2). ROS are expressed in various cellular compartments including peroxisomes, mitochondria and endoplasmic reticulum. These molecules play essential roles in cell signaling and homeostasis but can cause oxidative stress when present in excess, leading to cellular damage.

ROS Detection Assay Kit is based on the use of the fluorogenic substrate H_2DCFDA. H_2DCFDA is a cell-permeable, non-fluorescent compound that, once inside the cell, is deacetylated by intracellular esterase to H_2DCF. In the presence of ROS, H_2DCF is then oxidized to the highly fluorescent compound DCF (*dichlorofluorescein*). The intensity of DCF fluorescence can be measured providing a reliable indicator of intracellular ROS levels.

Features

- Highly sensitive assay.
- Fluorometric readout (Ex/ Em = 485/ 530 nm)
- Safe detection, non-radioactive compounds.
- High throughput.
- Versatile, suitable for adherent and suspension cells.

Kit Components

Components	TBK0530
H_2DCFDA Dye*	5 mg
Reaction Buffer 10x **	50 mL
Positive Control	500 µL
DMSO	1 mL

Order Info Kit Components: *H2DCFDA Dye (TBK0530-1) | Reaction Buffer 10x (TBK0530-2) | Positive Control (TBK0530-3) | DMSO (TBR0262).*

Before its use

* H_2DCFDA Stock Solution 20 mM: Add 500 µL DMSO to lyophilized substrate provided. Upon reconstitution, the stock solution should be stored in the dark at -20°C to -80°C. Avoid multiple freeze/thaw cycles.

H_2DCFDA Working Solution: Prepare a solution using Reaction Buffer 1x as diluent. The concentration would be in the range of 10 to 50 µM depending on the cell line to be used. Vortex well (15-30 secs) the mix obtained. This solution is unstable and must be prepared fresh.

** Reaction Buffer 1x: Add 10 mL Reaction Buffer 10x to 90 mL Water.

Storage

Store the kit at 2-8 °C.

Applications

- Measurement of intracellular levels of ROS.
- Fluorescence microscopy (*TRITC channel*).
- Flow Cytometry (*FL1 channel*).

Material required (not provided)

- Sterile black clear-bottomed 96-well plates (for microplate assays).
- Cell culture media without phenol red.

PROTOCOL

It is recommended to analyze samples/ controls in duplicate or triplicate.

I. ROS ASSAY (MICROPLATE)

Suspension Cells

1. Cultivate the cells to have approximately 1-2 x 10^5 cells per well in the assay day.
2. Collect the cells by centrifugation as usual and wash once in PBS 1x. Discard the supernatant.

Adherent Cells

1. Seed at a cell density of 2-3 x 10^4 cells per well the day previous to the assay and incubate overnight at 37°C, 5% CO2.
2. Remove the culture media and wash the cells with Reaction Buffer 1x and eliminate the supernatant.
3. Vortex gently **H_2DCFDA Working Solution** and add **100 µL** to the cells.
   Optimal concentration of H_2DCFDA Working Solution must be empirically determined.
4. Incubate at 37 °C for 30-60 minutes, in the dark.
5. Wash the cells in **Reaction Buffer 1x** and discard the supernatant.
6. Add **100 µL Test Compound / Positive Control/ Negative Control** per well.
7. Incubate at 37°C, protected from light, for experimentally determined time.
8. Read the plate at Ex/ Em 485/ 530 nm.

II. ROS ASSAY (MICROSCOPY)

1. Cultivate cells to ensure ~50-70% confluency.
   Avoid phenol red as it increases the background fluorescence signal. .
2. Wash adherent cells with Reaction Buffer 1x and eliminate the supernatant.
3. Vortex gently **H_2DCFDA Working Solution** and add **100 µL** to the cells.
   Optimal concentration of H_2DCFDA Working Solution must be empirically determined.
4. Incubate at 37°C for 30-60 minutes protected from light.
5. Remove H_2DCFDA Working Solution and wash the cells with Reaction Buffer 1x.
6. Discard Reaction Buffer 1x and applied the treatments. Incubate.
7. Visualize with fluorescence microscope using TRITC or FITC filter.

III. ROS ASSAY (CYTOMETRY)

1. Cultivate cells to have ~ 2-4 x 10^4 cells per test condition on the day of the assay.
2. Harvest the cells ensuring single cell suspension by pipetting or detaching adherent cells using trypsin.
3. Wash the cells with **PBS 1x**. Discard the supernatant.
4. Vortex gently **H_2DCFDA Working Solution** and add **100 µL** to the cells.
5. Incubate at 37°C for 30-45 minutes protected from light.
6. Remove H_2DCFDA Working Solution and wash the cells in **Reaction Buffer 1x**. Discard the supernatant.
7. Add **100 µL Test Compound / Positive Control/ Negative Control**.
8. Incubate at 37°C, protected from light, for experimentally determined time.
9. Analyze ~ 10,000 cells by flow cytometry using FL1 channel.