Reactive Oxygen Species Assay Kit

Product Information

Product name	Cat#	Size
Reactive Oxygen Species Assay Kit	50101ES01	1000 T

Product Description

Reactive Oxygen Species Assay Kit uses the cell permeant reagent 2',7'–dichlorofluorescin diacetate (DCFDA) to quantitatively assess reactive oxygen species in live cell samples. The lipophilic non-fluorescent DCFDA readily crosses the cell membrane through passive diffusion followed by deacetylation. The deacylated product is an oxidant sensitive 2′,7′-dichlorofluorescein (DCHF). DCHF is oxidized later by ROS to form DCF. DCF is highly fluorescent and is detected by fluorescence spectroscopy or Flow cytometry with excitation/emission at 480 nm/525 nm. Rosup, a compound mixture, is a ROS inducer and can be used as positive control.

Product Components

Component number	Components	Quantity	Storage
50101-A	DCFH-DA (10 mM)	100 μL	-20°C
50101-B	Rosup (100 mM)	1 mL	-20°C

Shipping and Storage

This kit is shipped with ice bag. Store at -20°C without light for 1 year. Avoid repeated freezing and thawing.

Method of application

1. Reagent Preparation

DCFH-DA Solution: Briefly centrifuge at low speed prior to opening. Prepare a working DCFH-DA solution by diluting 10 mM DCFH-DA in serum free medium to make a 10 μM final concentration.

[Note]: DCFH-DA may also be diluted in media without phenol red. Use freshly prepared DCFH-DA solution, long term storage of diluted DCFH-DA is not recommended. The exact concentration of DCFH-DA required will depend on the cell line being used but a general starting range would be 10- 50 μM. For certain cells, if the fluorescence of the negative control (without DCFH-DA probe) is very strong, dilute DCFH-DA to 2-5 μM and shorten the incubation time appropriately.

Rosup Solution: Prepare a 100 μM Rosup working solution by diluting 100 mM Rosup stock solution in serum free medium. Generally, incubation with Rosup at 37 ℃ for 30 min-4 h in dark can significantly increase ROS.

[Note]: The incubation time of Rosup will depend on the sensitivity of the cell line. For example, 30 min for Hela and 1.5 h for MRC5. If the increase of ROS is not observed within 30 minutes, the induction time or the concentration can be appropriately increased. If ROS rises too fast, the induction time or the concentration can be appropriately reduced.

Drugs: Prepare drug of interest in complete media with 10% FBS or other appropriate solution to desired concentration.

2. Recommended protocol for Adherent Cells

a) Cell preparation: Grow adherent cells in standard cell culture media the day before the experiment so that cell confluence reaches 70% at the time of experiment.

b) Drug induction: Remove the media. Overlay each well with previously prepared serum-free diluted drugs and incubate for desired time at 37°C in the dark.

c) (Optional) Positive control: Overlay the positive control well with previously prepared Rosup solution and incubate for desired

time at 37°C in the dark.

[Note]: For cells with short stimulation time (usually within 2 hours), the probe can also be loaded first, and then add Rosup or drug of interest.

d) ROS probe loading: Remove all the medium and wash cells with serum-free medium for 1-2 times. Overlay each well with previously prepared DCFH-DA Solution. Incubate at 37℃ for 30 min in the dark.

e) Remove the medium and wash cells 1-2 times with serum-free medium to remove free DCFH-DA.

3. Recommended protocol for Suspension Cells

a) Cell preparation: Grow suspension cells to approximately 1.5 × 10^5 cells per well on the day of the experiment.

b) Drug induction: Collect cells in a conical tube by centrifugation and resuspended in an appropriate amount of previously prepared serum-free diluted drugs and incubate for desired time at 37°C in the dark.

c) (Optional) Positive control: Resuspended the positive control cells with previously prepared Rosup solution and incubate for desired time at 37°C in the dark.

d) ROS probe loading: Collect in a new tube and wash cells by centrifugation twice in PBS. Resuspended the cells with previously prepared DCFH-DA Solution with cell density at 1×10^6-2×10^7/mL. Then incubate at 37℃ for 30 min in the dark. Invert the tube every 3-5 minutes to ensure fully contact between the probe and cells.

[Note]: The cell density should be adjusted according to the subsequent detection method. For example, for flow cytometry, the number of cells in a single tube should not be less than 10^4/mL or more than 10^6/mL.

e) Collect and wash cells by centrifugation twice with serum-free medium to remove free DCFH-DA.

4. Fluorescence detection and Data Analysis

Fluorescent microscopy Measurement: Perform live cell microscopy with filter set appropriate for fluorescein (FITC) using fluorescence microscope. Visually score cells for brightness and compare between control and samples or use image analysis methods to compare signal between on digital photographs of cells.

Flow Cytometry Measurement: The adherent cells should be collected with trypsin to prepare single cell suspension; For suspension cells, the cells are collected directly. Ideally 10,000 cells should be analyzed per experimental condition. Cells should not be overly dense during the experiment (<1 ×10^6 cells/mL). Exclude debris and isolate cell population ofinterest with gating. Using mean fluorescent intensity, determine fold change between control and treated samples with Ex/Em = 480/525 nm.

Fluorescent Microplate Measurement: Measure plate immediately on a fluorescence plate reader at Ex/Em = 480/525 nm in end point mode in the presence of media. Subtract blank readings from all measurements and determine fold change from assay control.

Cautions

1. Wash the cells after incubating with DCFH-DA to reduce background noise.
2. It is recommended to measure the fluorescence as soon as possible after incubation to avoid possible errors.
3. For your safety and health, please wear lab coats and disposable gloves for operation.
4. For research use only!

Product Citations

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