Reactive Oxygen Species Assay Kit

Introduction

Reactive Oxygen Species Assay Kit is a kit that uses the fluorescent probe DCFH-DA to quantitatively assess reactive oxygen species (ROS) in live cells. DCFH-DA is a cell-permeable probe, which is deacetylated by cellular esterases to a non-fluorescent DCFH. DCFH then is oxidized by ROS into highly fluorescent DCF. The fluorescence intensity of DCF is proportional to the ROS levels. This kit also provides Rosup as a positive control for ROS generation. The concentration of Rosup is 50 mg/mL.

Components and Storage

Components	K2065-100 T	K2065-500 T
DCFH-DA (10 mM)	0.1 mL	0.5 mL
Rosup	1 mL	5 mL
This kit should be stored at -20°C away from light, stable for 1 year, and avoid repeated freeze/thaw cycles.		

Protocol

For a short drug stimulation time (< 2 h), it is recommended to load the probe first, and then stimulate the cells with the interested drug or Rosup; For a long drug stimulation time (> 6 h), it is recommended to stimulate the cells with the interested drug or Rosup first. Here, the second method is used as an example.

1. **Preparation of DCFH-DA working solution:** dilute DCFH-DA (10 mM) in serum-free medium to make a 10 μM working solution. The working solution is unstable, any unused working solution should be discarded after use.

***Note:** The optimal concentration of working solution varies depending on the cell types. The recommended concentration of working solution can be in the range of 1-10 μM.

2. **Loading of DCFH-DA probe:**

A. For adherent cells

If adherent cells need to be detected by a fluorescence spectrophotometer or flow cytometer, the cells can be harvested and digested first, and then suspended and operated according to the steps for suspended cells.

1) **Drug induction:** Treat the experimental group cells with the interested drug. The time of drug stimulation

varies depending on the specific drug used.

2) **Positive control (optional):** Dilute the Rosup with serum-free culture medium at a ratio of 1:1000 (1 mL serum-free culture medium + 1 μL Rosup) to make a positive control working solution. For the positive control group, remove the cell culture medium and add the positive control working solution to induce ROS in the cells.

***Note:** For 6-well plates, at least 1 mL of the positive control working solution is needed for each well. When Rosup is used at a 1:1000 ratio, the level of ROS can be significantly increased within 20-30 minutes.

3) **Probe loading:** Remove the cell culture medium, wash the cells 1-2 times with serum-free culture medium, and then add an appropriate amount of DCFH-DA working solution to each well to fully cover the cells. Incubate at 37°C in the dark for 30 minutes, shaking every 5 minutes to ensure the probe fully contacts the cells.

***Note:** For 6-well plates, at least 1 mL of the positive control working solution is needed for each well. The probe incubation time can be adjusted according to the specific experiment.

4) **Washing:** After incubation, wash the cells several times with serum-free culture medium, add an appropriate amount of PBS or other suitable buffer, and then perform in situ detection using a fluorescence microscope.

B. For suspended cells

1) **Drug induction:** Treat the experimental group cells with the interested drug. The time of drug stimulation varies depending on the specific drug used.

2) **Positive control (optional):** Dilute the positive control Rosup with serum-free culture medium at a ratio of 1:1000 (1 mL serum-free culture medium + 1 μL Rosup) to make a positive control working solution. For the positive control group, centrifuge to remove the cell culture medium, add an appropriate amount of positive control working solution to resuspend the cells and induce ROS.

***Note:** When Rosup is used at a 1:1000 ratio, the level of ROS can be significantly increased within 20-30 minutes.

3) **Probe loading:** Centrifuge to remove the treatment drug, wash the cells 1-2 times with serum-free culture medium. Centrifuge to collect the cells, then add an appropriate amount of DCFH-DA working solution to resuspend the cells to a cell density of 1×10^6 -2×10^7 cells/mL. Incubate at 37°C in the dark for 30 minutes, inverting every 5 minutes to ensure the probe fully contacts the cells.

***Note:** The probe incubation time can be adjusted according to the specific experiment.

4) **Washing:** After incubation, wash cells several times with serum-free culture medium, and add an appropriate amount of PBS or other suitable buffer to resuspend the cells. Then use a fluorescence spectrophotometer, fluorescence microplate reader, or flow cytometer for detection. Alternatively, an appropriate amount of cell suspension can be taken to make a smear for detection with a fluorescence microscope.

3. **Detection:** Measure the fluorescence of DCF using a 488 nm excitation wavelength and a 525 nm emission wavelength or directly use the FITC filter set.

Note

1. DCFH-DA should avoid repeated freeze/thaw cycles. So, it is better prepared in single-use aliquots.
2. After the probe incubation is completed, make sure to remove excess probes as much as possible, otherwise the background fluorescence will be high.
3. DCF fluorescence is easy to quench. Therefore, the time required from probe incubation to detection should be shortened as much as possible to avoid poor detection results.
4. This kit is suitable for ROS detection in live cells. For fresh tissue, it can be prepared into a single-cell suspension for detection.
5. When Rosup is used at 1:1000 dilution, the level of ROS can be significantly increased within 20-30 minutes. Meanwhile, the optimal concentration and incubation time vary depending on cell types.
6. The optimal concentration of DCFH-DA varies depending on cell types. If the fluorescence of the negative control is also strong, the DCFH-DA can be diluted at 1:2000-1:5000 to make the final concentration of 2-5 μM.
7. For your safety and health, please wear lab coats and gloves during the experiment.
8. For research use only. Not to be used in clinical diagnostic or clinical trials.

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