Reactive Oxygen Species (ROS) Assay Kit

Catalog No: abx295093

Size: 96T

Storage: Store DCFH-DA (10 mmol/L) at -20°C in the dark, and the remaining components at 2-8°C.

Application: The detection of Reactive Oxygen Species (ROS) in cell and tissue samples.

Principle of the Assay: This kit is based on fluorescence technology. DCFH-DA is a fluorescent precursor that can cross cell membranes. After entering cells, it is hydrolysed by intracellular esterase to form DCFH. In the presence of ROS, DCFH is oxidized to fluorescent DCF, which cannot cross cell membranes. The intensity of the color green is directly proportional to intracellular ROS, which can be determined with a fluorescence microplate reader (Excitation/Emission - 500 nm / 525 nm).

Kit Components

- Black 96-Well Microplate: 2 x 96
- DCFH-DA (10 mmol/L): 0.1 ml
- Positive Control: 1 ml
- Plate Sealer: 4

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Double distilled water
- PBS (0.01 M, pH 7.4)
- Serum-free medium
- FBS-supplemented medium
- Water bath
- Vortex mixer
- Fluorescence microplate reader (wavelength Excitation/Emission - 500 nm / 525 nm)
- Microplate Shaker

For Reference Only

Protocol

A. Reagent Preparation

DCFH-DA Working Solution: Dilute DCFH-DA (10 mmol/L) with serum-free medium to a working concentration of 0.1 µmol/L – 20 µmol/L. Prepare immediately before use. The dilution ratio must be greater than 500-fold.

Positive Control Working Solution: The positive control contains 10 mmol/L TBHP. Dilute the Positive control with serum-free medium 40 to 200-fold to a TBHP working concentration of 50 µmol/L – 250 µmol/L.

B. Assay Protocol

Cell samples

1. **Sample:** Add DCFH-DA Working Solution to the cell samples. The Working Concentration of DCFH-DA will differ between cell type and treatment, and a pre-experiment is recommended to determine optimal concentration.
2. **Negative control:** Prepare cell samples without adding DCFH-DA. Each sample requires a negative control.
3. Incubate at 37°C for 30 – 60 minutes. The incubation time is for reference only, and the optimal time should be determined by the end user dependent on the cell type, treatment, and DCFH-DA concentration.
4. Cell collection:
   Suspension cells: Centrifuge the sample at 1000 × g for 10 minutes. Discard the supernatant, resuspend and wash the pellet with serum-free medium, and centrifuge again. Wash the cell pellet and centrifuge a total of 3 times. Collect the cell pellet, and resuspend in serum-free medium for detection.
   Adherent cells: Detach adherent cells with 0.25% trypsin, and add FBS-supplemented medium. Centrifuge the sample at 1000 × g for 10 minutes. Discard the supernatant, resuspend and wash the cell pellet with serum-free medium, and centrifuge again. Wash the pellet and centrifuge a total of 2 times. Collect the cell pellet, and resuspend in serum-free medium for detection.
5. Set sample, blank, positive control and negative control wells on the 96-well microplate, and record their positions.
6. Aliquot DCFH-DA Working Solution only to the blank wells.
7. Aliquot the samples, blank, positive control and negative control to respective wells on the microplate.
8. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Read and record the absorbance with a fluorescence microplate reader (excitation – 500 nm; emission – 525 nm).

Tissue samples

1. **Sample pretreatment:** Collect tissue into pre-cooled PBS (0.01 M, pH 7.4). Wash with pre-cooled PBS to remove blood and other contaminants. Remove any fat, fibrous tissue and blood vessels. Cut tissue into small pieces, and wash with PBS to remove cell debris. Tissue samples may be prepared with enzyme digestion *or* mechanical homogenization:
   Enzyme digestion: Add enzyme to the tissue, mix fully and incubate at 37°C for 20-30 minutes, mixing intermittently. Add FBS-supplemented medium. Filter the mixture with 300 nylon mesh. Centrifuge at 500 × g for 10 minutes and discard the supernatant. Wash the cell pellet with serum-free medium, and centrifuge again. Wash the pellet and centrifuge a total of 2 times. Collect the cell pellet, and resuspend in serum-free medium for detection.
   Mechanical homogenization: Cover a small beaker with 300 nylon mesh. Place the tissue pieces on the mesh, and scrape the tissue along the mesh with a knife or scissor, whilst washing with PBS. . Centrifuge at 500 × g for 10 minutes and discard the supernatant. Wash the cell pellet with serum-free medium, and centrifuge again. Wash the pellet and centrifuge a total of 2 times. Collect the cell pellet, and resuspend in serum-free medium for detection.
2. **Sample:** Add DCFH-DA Working Solution to the cell samples. The Working Concentration of DCFH-DA will differ between cell type and treatment, and a pre-experiment is recommended to determine optimal concentration.
3. **Negative control:** Prepare samples without adding DCFH-DA. Each sample requires a negative control.
4. Incubate at 37°C for 30 – 60 minutes. The incubation time is for reference only, and the optimal time should be determined by the end user dependent on the cell type, treatment, and DCFH-DA concentration.
5. Centrifuge at 1000 × g for 10 minutes. Discard the supernatant, resuspend and wash the pellet with serum-free medium, and centrifuge again. Wash the cell pellet and centrifuge a total of 3 times. Collect the cell pellet, and resuspend in serum-free medium for detection.
6. Set sample, blank, positive control and negative control wells on the 96-well microplate, and record their positions.
7. Aliquot DCFH-DA Working Solution only to the blank wells.
8. Aliquot the samples, positive control and negative control to respective wells on the microplate.
9. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Read and record the absorbance with a fluorescence microplate reader (excitation – 500 nm; emission – 525 nm).

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Notes:

- Final cell density should be determined dependent on the fluorescence intensity. If fluorescence is too strong, decrease cell density. If fluorescence is too weak, increase cell density. Cell density should be at least 10^6 cells per ml.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.
- Samples should be measured within 2 hours of incubation.
- Samples should be free of precipitation or cell debris prior to detection.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled. Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection in other sample types.
- This kit is for research use only.

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