Mitochondrial Membrane Potential and Apoptosis Detection Kit

Introduction

Annexin V is a Ca^{2+}-dependent phospholipid-binding protein that binds specifically to phosphatidylserine (PS). PS is normally located medial to the lipid bilayer of the cell membrane, but in the early stages of apoptosis, PS can be flipped from the medial side of the cell membrane to the outer surface of the cell membrane, where it is exposed to the extracellular environment. Annexin V has a high affinity for PS, and the occurrence of apoptosis can be detected by fluorescein labeling with Annexin V.

The Mito-Tracker Red CMXRos, also known as MitoMark Red I, is an oxidized red fluorescent probe that specifically labels mitochondria. Mito-Tracker Red CMXRos can cross the cell membrane of living cells and aggregate specifically on the mitochondria of the cell. When the mitochondrial membrane potential decreases, the red fluorescence signal of the Mito-Tracker Red CMXRos also decreases, and the mitochondrial damage can be judged based on the strength of the fluorescent signal.

Mitochondrial Membrane Potential and Apoptosis Detection Kit is a kit for detecting apoptosis and mitochondrial membrane potential by the apoptosis green fluorescent probe Annexin V-FITC and the mitochondrial membrane potential red fluorescent probe Mito-Tracker Red CMXRos. Hoechst 33342 is also available for staining nuclei. After staining of this kit, the live cells are positive for red fluorescence and negative for green fluorescence; Apoptotic cells, on the other hand, are positive for green fluorescence and significantly weakened or negative for red fluorescence.

Components and Storage

Components	K2264-20T	K2264-50T
Mito-Tracker Red CMXRos (10X)	6 μL	12 μL
Annexin V-FITC	200 μL	500 μL
Annexin V-FITC Binding Buffer	12 mL	30 mL
Hoechst 33342	100 μL	250 μL
Store Annexin V-FITC at 4°C away from light, stable for 6 months. Store Other reagents at -20°C, stable for 1 year, Mito-Tracker Red CMXRos (10X) and Hoechst 33342 should be stored away from light.		

Protocol

1. Induction of apoptosis by the desired method.

2. Collect cells.

    1) For suspension cells: 1000 rpm centrifugation for 5 min, and the supernatant of the medium is discarded.

    2) For adherent cells: Try to use EDTA-Free trypsin to digest cells, 1000 rpm centrifugation for 5 min, and the supernatant is discarded.

3. Wash cells with pre-chilled PBS and collect 1-5×10^5 cells.

4. Resuspend cells in 186 μL of Annexin V-FITC Binding Buffer.

5. Dilute Mito-Tracker Red CMXRos (10X) in PBS to make Mito-Tracker Red CMXRos (1X).

6. Add 2 μL of Mito-Tracker Red CMXRos (1X), 10 μL of Annexin V-HF488 and 2 μL of Hoechst 33342. Mix gently and incubate at room temperature for 20-30 min in the dark. Resuspending cells every 5 min during incubation is recommended to improve staining.

*Note: Hoechst 33342 staining is optional and can be added depending on the experiment. The concentration of the fluorescent probes can also be adjusted to obtain better staining.

7. After incubation, 1000 rpm centrifugation for 5 min, and the supernatant of the medium is discarded. Resuspend cells in Annexin V-FITC Binding Buffer.

8. Detection must be performed with flow cytometry or fluorescence microscopy as soon as possible. If testing is not possible immediately, place the sample on ice and test within 1 h.

    1) Flow Cytometry: Annexin V-FITC is green fluorescent (Ex/Em: 490/525 nm), Mito-Tracker Red CMXRos is red fluorescent (Ex/Em: 579/599 nm), and Hoechst 33342 is blue fluorescent (Ex/Em: 350/461 nm)

    2) Fluorescence Microscopy: Add 30-50 μL of the cell suspension from step 7 dropwise to a glass slide, cover the cells with a coverslip, and observe under fluorescence microscopy.

Note

1. Since apoptosis is a rapid process, it is recommended to complete the analysis within 1 h after staining. Otherwise, it may lead to an increase in the number of apoptosis or necrotic cells.

2. During the whole operation, the action should be as gentle as possible, and the cells should not be pipetted forcefully to avoid mechanical damage to the cells.

3. If the sample is derived from blood, be sure to remove platelets from the blood. Because platelets contain PS, they can bind to Annexin V, which can interfere with the results. Platelets can be washed using a buffer containing EDTA and centrifugation at 1400 rpm (200×g).

4. Please centrifuge the reagent briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when the cap is opened.

5. Mito-Tracker Red CMXRos, Annexin V-FITC and Hoechst 33342 are photosensitizing substances, please take care to protect them from light when handling.
6. Mito-Tracker Red CMXRos and Hoechst 33342 are known as mutagens. So be careful when using it.
7. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the same time, the digestion fluid of trypsin should be EDTA-free as much as possible, because EDTA will affect the binding of Annexin V to PS. If EDTA-containing trypsin is used, the cells should be washed thoroughly after collection to ensure that the EDTA is cleanly removed.
8. For your safety and health, please wear lab coats and gloves during the experiment.
9. For research use only. Not to be used in clinical diagnostic or clinical trials.

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