In Situ Fluorescein TUNEL Cell Apoptosis Detection Kit

Catalog No.: abx098881

Size: 25 tests / 50 tests

Storage: Store at -20° C in the dark for 12 months.

Application: For detection and quantification of apoptotic cell death at the single cell level in cells and tissues, including paraffin-embedded tissue sections, cryopreserved tissue sections, cells cultured on chamber slides, cell smear and cell suspensions.

Introduction

Apoptosis is a regulated process of cell death that occurs during embryonic development as well as maintenance of tissue homeostasis. Inappropriately regulated apoptosis is implicated in different disease states, such as neurodegeneration disease and cancer. The apoptosis program is characterized by morphologic features, including loos of plasma membrane asymmetry and attachment, condensation off the cytoplasm and nucleus, and compaction and fragmentation of the nuclear chromatin. In normal viable cells, phospholipids are asymmetrically distributed between inner and outer leaflets of the plasma membrane whit phosphatidylcholine and sphingomyelin exposed on the external leaflet of the lipid bilayer, and phosphatidylserine predominantly observed on the inner surface facing the cytosol.

Principle of the Assay

TdT-mediated dUTP Nick-End Labeling (TUNEL) reaction preferentially labels DNA strand breaks generated during apoptosis with fluorescein-labeled dUTP. The fluorescein labeled DNA can be detected and quantified by fluorescence microscopy or flow cytometry.

Kit components

1. TdT: 50 µl (25 tests), 100 µl (50 tests)
2. 1x Labelling solution: 1x 1.25 ml (25 tests), 2x 1.25 ml (50 tests)

Material Required But Not Provided

1. Formaldehyde fixing solution（3% formaldehyde and 2% sucrose in PBS）
2. Permeabilisation solution（0.1% Triton X-100 in PBS）
3. DNase I (abx098138), 10× DNase I Reaction Buffer
4. Anti-Fade solution
5. Double-distilled water (ddH2O)
6. Ethanol
7. Phosphate-buffered saline (PBS)

Protocol

A. Paraffin-embedded tissue

1. **Dewax and rehydrate:** Dewax by washing in xylene and rehydrate through a series of gradient concentrations of ethanol (e.g. 95%, 90%, 85%, 75%, 50%) and double-distilled water. Then rinse the slides with PBS and carefully remove liquid around the section. Ensure that the surface of the slides are kept moisturised.
2. **Permeabilisation:** Apply 100 µl of Permeabilisation solution to the slides, then incubate at room temperature for 5 minutes.
3. **Labeling:** Mix 50 µl of 1× Labeling Solution and 2 µl of TdT thoroughly (ratio of 25:1). Then apply to the slide surface. Incubate at 37 °C in the dark for 1 hour.
   Note: The volume of 1× Labeling Solution can be adjusted according to the sample size. Labeling solution should cover the whole sample; insufficient liquid could result in labeling failure. Use a humidified chamber to keep the slide moisturised. Starting from this step, avoid exposure to light to prevent fluorescence quenching.
4. Rinse with Permeabilisation solution 3 times, for 5 minutes each time.
5. Carefully remove liquid around the section. Add appropriate volume of Anti-Fade solution. The samples are now ready to be analysed by flow cytometry or fluorescent microscope.

B. Cryopreserved tissue

1. **Fixation:** For unfixed sections, use Formaldehyde fixing solution to fix at room temperature for 30 minutes. Rinse with PBS 3 times, for 5 minutes each time. For fixed sections, this step can be skipped.
2. Same as steps 2-5 in paraffin-embedded tissue section (Section A).

C. Fixed cell slides

1. Prepare adherent cells on slides or cell smear.
2. **Fixation:** Use Formaldehyde fixing solution to fix at room temperature for 30 minutes. Rinse with PBS 3 times, for 5 minutes each time.
3. **Permeabilisation:** Add enough Permeabilisation solution to immerse the slides. Incubate at room temperature for 5 minutes.
4. Same as steps 3-5 in paraffin-embedded tissue section (Section A).

D. Cell suspensions

1. Centrifuge the suspension at 500 × g for 5 minutes. Discard the supernatant, rinse the pellet with PBS twice. Centrifuge at 500 × g for 5 minutes. The optimal number of cells is approximately 1×10^5 cells.
2. **Fixation:** Add enough formaldehyde fixing solution, then carefully re-suspend cells by inversion. Incubate at room temperature for 30 minutes. Re-suspend by inverting 2-3 times during incubation.
3. Rinse with PBS 3 times, centrifuge at 500 × g for 5 minutes. Discard the supernatant.
   Note: Rinse, gently invert and re-suspend cells before centrifugation.
4. **Permeabilisation:** Add enough permeabilisation solution, gently re-suspend cells and incubate at room temperature for 10 minutes. Re-suspend by inverting 2-3 times during incubation.
5. **Labeling:** Centrifuge at 500 × g for 5 minutes, then discard the supernatant. Mix 50 µl of 1× Labeling Solution

and 2 µl of TdT thoroughly (ratio of 25:1) and then add the mixture into the cells. Incubate at 37 °C in the dark for one hour (re-suspend by inverting several times during incubation).

6. Centrifuge at 500 × g for 5 minutes, then discard the supernatant. Rinse the pellet with Permeabilisation solution twice and re-suspend with PBS. Detect by flow cytometry or fluorescent microscope.

E. Positive control

1. Treat the positive control for permeabilisation as described above. Dilute 10× DNase I Reaction Buffer with permeabilisation solution to 1× and then add DNase I (final concentration at 10-15 U/ml). Mix thoroughly and add to the positive control. Incubate at room temperature for 15-30 minutes.
2. Rinse positive control with permeabilisation solution 3 times, for 5 minutes each time.
3. Same as steps 3-5 in paraffin-embedded tissue section (Section A).