(FITC) Tunel Assay Protocol

Frozen-slides

(For scientific research purposes only, not for clinical diagnosis!)

Procedure

1 Fix frozen-slides: Restore the frozen slices in an oven at 37℃ for 10-20min, and control the moisture. Fixing in 4% paraformaldehyde for 30min, shaking and washing in PBS(PH7.4) on a decoloring shaker for 3 times, 5min each time.

2 Antigen retrieval: Dry the slices slightly , then draw a circle on the position where the cells are evenly distributed in the middle of the cover glass with a Liquid Blocker PAP Pen (to prevent the antibody from flowing away), add protease K working solution to cover the tissue, and incubate at 37℃ for 22min. Wash the slide with PBS(PH7.4) in a decoloring shaker for 3 times, 5 minutes each time. (Preparation method of working solution of protease K, stock solution: PBS=1:9)

3 Permeabilization: Dry the cell slide slightly , then add permeabilize working solution to cover the tissue, incubate at room temperature for 20min, and wash with PBS for 3 times for 5 min each time.(The permeabilize working solution is 0.1% triton. Configuration method, triton stock solution: PBS=1:1000)

4 Equilibrium at room temperature: After the slice was slightly dried, buffer was dripped into the circle to cover the tissue, and the buffer was incubated at room temperature for 10min.

5 Tunel reaction: Take appropriate amount of TDT enzyme, dUTP and buffer in the tunel kit according to the number of slices and tissue size and mix at 1:5:50 ratio ,and added to the circle to cover tissue.in a flat wet box, incubate at 37℃ for 1 h. be sure to keep the wet box moist by adding water.

6 DAPI counterstain in nucleus: Wash three times with PBS (pH 7.4) in a decoloring shaker , 5min minutes each time. After removing PBS, DAPI solution was dripped into the circle and incubated at room temperature for 10min in the dark.

7 Mount: Wash three times with PBS (pH 7.4) in a decoloring shaker , 5min minutes each time. After the climbing slide is slightly dried, then coverslip with anti-fade mounting medium.

8 Microscopic examination and collecting images through fluorescence microscope. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; FITC glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm.

Results

The nuclei stained by DAPI are blue under the excitation of ultraviolet light. Tunel assay kit is labeled with FITC. Positive apoptotic nuclei are green.