Immunofluorescence Staining Protocol

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

Procedure

1. Deparaffinize and rehydrate: Put the slices in 3 changes of xylene, 10min each, then dehydrate in 3 changes of pure ethanol for 5min each, wash in distilled water.
2. Antigen repair: See the above table for repair conditions. During the repair process, excessive evaporation of buffer solution should be prevented, and the slides should not be dried. After the repair is completed, it is naturally cooled. Put the slide 5min PBS(PH7.4) and shake it on a decoloring shaker for 3 times, each time for 5 minutes.
3. Circle drawing and blocking: Add 3%BSA into the circle and cover the tissue evenlyto block non-specific binding at room temperature for 30 minutes.(The primary antibody is blocked with 10% donkey serum from goat, and the primary antibody from other sources is blocked with 3%BSA).
4. Adding primary antibody: drop the prepared primary antibody, slice it flat in a wet box and incubate at 4℃covernight.
5. Add secondary antibody: put the glass slide 5min PBS(PH7.4) and shake it on the decoloring shaker for 3 times, 5 minutes each time. Add the corresponding secondary antibody and incubate at room temperature for 50min in the dark.
6. DAPI counterstain in nucleus: DAPI solution was dripped into the circle and incubated at room temperature for 10min in the dark.
7. Quench tissue autofluorescence:The slides were put 5min PBS(PH7.4) and washed on a decoloring shaker for 3 times, each time for 5 minutes. Add autofluorescence quencher B solution for 5min and rinse with running water for 10min.
8. Mount: coverslip with anti-fade mounting medium.
9. Microscopy detection and collect images by Fluorescent Microscopy. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; 488 glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm; CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nm.

Results

The nucleus of DAPI channel is blue,the positive of 488 channel is green, CY3 channel is red and CY5 channel is pink.