Immunofluorescence Microscopy Protocol with Methanol Fixed Cells(about S100A6 in SW480 Cells)

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Abstract

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Protocol

Sample preparation

Step 1.

1. The cells were grown on coverslips for 2 days in DMEM at 37°C and treated with corresponding pretreatment. At the time of fixation, cells should be 50% confluent.
2. Rinse cells briefly in 1X PBS.
3. Fix cells with 4% cold paraformaldehyde for 20 min at room temperature.
4. permeabilized for 10 min with 0.3% Triton X-100 at 37 °C.
5. Rinse three times in 1X PBS, 5 minutes each.
6. Block samples in 5% normal goat serum for 30 min at room temperature.

Sample staining

Step 2.

7.Dilute the primary antibody to the recommended concentration/dilution in 5% FBS/PBS overnight at 4 ℃.

8.Rinse three times in 1X PBS, 5 minutes each.

9.Prepare FITC-conjugated secondary antibody in 5% FBS/PBS in the dark for 1 hour at room temperature according to the recommended manufacturer specification data sheet.

10.Nuclei were stained with DAPI for 5 min.

11.Rinse three times in 1X PBS, 5 minutes each.

12.Coverslip with anti-fade mounting medium.

13.Seal the edges of the coverslip to the slide to prevent movement of the coverslip while imaging. One sealant that is recommended is a 1:1:1 ratio mixture of vasoline, lanolin and paraffin.

14.Immunofluorescence was analyzed with a META-510 Laser Scanning Confocal Imaging System.

Material and Buffers

Step 3.

15.Chamber slides and coverslips.

16.Antibody dilution solution: 5% FBS in 1X PBS