Immunocytochemistry Staining Protocol

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Abstract

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Before start

Reagent List:

- Chamber slides, cover slips, or 12-well plates
- Phosphate-buffered saline (PBS)
- Fixation solution: 1% Paraformaldehyde, in PBS
- Permeabilization solution: 0.5% Triton X-100 in PBS
- Blocking buffer: 5% FBS in PBS

Protocol

Sample Preparation

Step 1.

Grow cultured cells on cover slips or in wells overnight at 37°C. At the time of fixation, cells should be 70-80% confluent in single layer.

Sample Preparation

Step 2.

Rinse cells briefly in PBS.

Sample Preparation

Step 3.

Fix cells by incubation with freshly made 4% Paraformaldehyde in PBS for 15 minutes at room temperature.

DURATION
00:15:00 :

Sample Preparation

Step 4.

Rinse three times quickly in PBS.

Sample Blocking

Step 5.

Block samples in 1 mL of blocking buffer at room temperature for 1 hour.

DURATION

00:30:00 :

Sample Staining

Step 6.

Dilute the primary antibody to the recommended concentration/dilution in blocking buffer.

Sample Staining

Step 7.

For 8-well chamber slides, add 200 µL per well. For 12-well plates, add 500 µL per well. Incubate two to three hours at room temperature or overnight at 4°C. If using conjugated antibodies, perform this step in the dark.

Sample Staining

Step 8.

For surface staining, rinse 3 times quickly in PBS.

DURATION

00:10:00 :

Sample Staining

Step 9.

Prepare fluorochrome-conjugated secondary antibody in blocking buffer according to the manufacturer's specification data sheet, and add 200 µl per well to the 8-well chamber slides. For 12-well plates, add 500 µL per well.

Sample Staining

Step 10.

Incubate the samples for one hour, at room temperature, in the dark.

DURATION

01:00:00 :

Sample Staining

Step 11.

For surface staining, rinse three times quickly in PBS.

DURATION

00:10:00 :

Sample Staining

Step 12.

Counterstain with DAPI for 15 minutes at 37 ℃.

Sample Staining

Step 13.

Wash cells 3 times and the third time keep PBS in the wells.

Sample Staining

Step 14.

Image CD31 at 488nm and DAPI at 388nm