ICC ARv7 Protocol

Reagents

1. Phosphate-buffered saline (PBS, pH 7.4).
2. Fixative solution (4% paraformaldehyde in PBS, pH to 7.0 is necessary).
3. Permeabilization buffer (0.5 % Triton X-100 in PBS).
4. Blocking buffer (5% Goat Serum in PBS with 0.05% NaN3).
5. Antibody dilution buffer (1%BSA in PBS with 0.05% NaN3).
6. Primary antibody (RevMAb rabbit monoclonal antibody).
7. Anti-rabbit secondary antibody (Alexa Fluor 594).
8. Fluorescein Phalloidin staining buffer.

Method

1. Fix cells with Fixative solution for 10 min at RT.
2. Wash the cells once with PBS at RT for 30 s.
3. Permeabilize the cells with Permeabilization buffer for 5 min at RT.
4. Wash the cells twice with PBS at RT for 30 s.
5. Block with Blocking buffer for 1 hr at RT.
6. Incubate with the primary antibody (diluted in Antibody dilution buffer) for 1-2 hr at RT.
7. Wash 3 times in PBS at RT for 5 min each wash.
8. Incubate with the secondary antibody (diluted in Antibody dilution buffer) for 1 hr at RT.
9. Wash 3 times in PBS at RT for 5 min each wash.
10. Stain actin filaments with Fluorescein Phalloidin staining buffer.
11. Wash the coverslip 3 times in PBS for 30 s each wash.