Immunofluorescence (Indirect Staining) Protocol for Adherent Cells

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[Abstract] In this protocol, different types of adherent cells are fixed to coverslips by 4% paraformaldehyde. Target proteins are then stained by specific antibodies and fluorescent 2nd antibodies. This protocol therefore provides a method for immunostaining of adherent cells.

Materials and Reagents

1. Raw264.7, MCF-7 or Hela cells
2. DPBS (Life Technologies, Invitrogen™, catalog number: 14190-250)
3. FBS (Atlanta Biologicals, catalog number: S11110H)
4. Anti-fade mounting medium: *e.g.* ProLong Gold Antifade Mountant (Life Technologies, Invitrogen™, catalog number: P10144) with DAPI (if nuclear staining is needed) or without DAPI
5. Paraformaldehyde (Sigma-Aldrich, catalog number: 158127)
6. General chemicals (Sigma-Aldrich)
7. Triton X-100 (Sigma-Aldrich, catalog number: T8787)
8. Tween 20 (Sigma-Aldrich, catalog number: P2287)
9. 4% paraformaldehyde-freshly-prepared (5 ml) (see Recipes)
10. Antibodies (see Recipes)

Equipment

1. Round cover slips (Thermo Fisher Scientific)
2. 24-well plate
3. Fluorescence microscope

Procedures

1. Grow cells on round cover slips in 24-well plates to 30-60% confluence. Do not overgrow because it will be difficult to distinguish cell components when stained.

2. Wash cells with 500 µl/well DPBS twice, then add 250 µl/well 4% paraformaldehyde for 15 min at room temperature (RT).
3. Remove paraformaldehyde, wash the fixed cells with 500 µl/well PBS + 3% FBS for 3 times.
4. Permeabilize cells with 250 µl/well DPBS + 0.2% Triton X-100 for 5 min, then wash with 500 µl/well DPBS + 3% FBS for 3 times.
5. Block with 500 µl/well DPBS + 3% FBS + 0.5% Tween 20 for 1 h at RT.
6. Remove the blocking buffer, add 250 µl/well primary antibodies and incubate at RT for 1 h.
7. Remove the antibodies, wash with 500 µl/well DPBS + 3% FBS in 5 min 3 times.
8. Add 250 µl/well fluorescent secondary antibodies and incubate at RT for 30 min.
9. Remove the antibodies, wash with 500 µl/well DPBS + 3% FBS in 5 min for 3 times.
10. Place a small drop of anti-fade reagent on a glass slide, then get the cover slip from the well and put it face down on the drop, push it tightly and attach to the glass slide.
11. Leave the slide in the dark for 5 min to let it dry.
12. The slide is ready to observe under a fluorescence microscope.

Recipes

1. 4% paraformaldehyde-freshly-prepared (5 ml)
   0.2 g paraformaldehyde powder + 5 ml DPBS + 50 µl 1 N NaOH
   Incubate at 65 °C
   Vortex several times to dissolved completely
   Cool at room temperature then add 4 µl HCl
   Mixed completely
2. Antibodies
   Diluted in DPBS + 3% FBS + 0.5 Tween 20
   Primary antibodies: 1:100-1:500 (depends on individual antibodies)
   Fluorescent secondary antibodies: 1:800

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References

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