ABGENT CUSTOM SERVICES:

Immunofluorescence Protocol

Step-by-step procedure:

1. Add a coverslip into a 12-well plate and grow cells in culture media until they reach 50% confluence.
2. Aspirate media from plates and wash twice with PBS.
3. Fix cells with 4% paraformaldehyde solubilized in 0.1% Triton ×100-PBS for 20 min at room temperature (RT).
4. Block for 1 hr with 2 ml of 1% BSA-4% goat serum-PBS. (Note: always spin down any sera, antibodies, or antisera for 5 min at 10,000 g before use, to remove small aggregates)
5. Wash twice for 5 min with 2 ml of PBS.
6. Stain with primary antibody for 45 min at RT in 40 ml of 1%BSA-PBS by forming a drop on the coverslip.
7. Wash twice for 5 min with 0.2% BSA-PBS.
8. Stain with conjugated secondary antibody for 30 min at RT in 40 ml of 1% BSA-PBS.
9. Wash twice for 5 min with 2 ml of PBS.
10. Mount slide with anti-fading agent.

Reagents:

PBS（pH7.4）:
10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 50 mM NaCl, 2.7 mM KCl

Fixative preparation:

4% paraformaldehyde solubilized in PBS:
Depolymerize paraformaldehyde by adding 1-2 drops of 10N NaOH/25 ml and warm the tube up to 65 ℃ to get a clear solution, put back on ice and check the pH.

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