Protocol

General Protocol for Immunocytochemistry

OVERVIEW

The following procedure is a general protocol for immunocytochemistry (ICC) of fixed cells. The reagent amounts listed below are intended for use in a 24-well format. Amounts suggested can be scaled up or down, depending on plate format and well size.

REQUIRED MATERIALS

- 24-well tissue culture plates
- PBS (without Mg^{2+} or Ca^{2+})
- 4% Paraformaldehyde in PBS
- Triton X-100
- Animal serum
- DAPI (optional)
- Mounting medium (optional)

MATERIAL PREPARATION

- **Preparation of Blocking Buffer:** In an appropriate sized tube or bottle, add the amount of PBS necessary to complete the ICC. Add 10% of serum from the same species as the secondary antibody used in the ICC procedure.
    - If detecting **surface antigens**, the Blocking Buffer is ready for use.
    - If detecting **intracellular antigens**, add 0.1% of Triton X-100.

  Discard any remaining Blocking Buffer.

ICC PROCEDURE

1. Seed and culture cells in a 24-well plate until ready for ICC analysis.
2. Wash each well 3 times with 0.5 ml of room temperature PBS.
3. Fix each well by adding 0.5 ml of 4% paraformaldehyde in PBS and incubating for 20 minutes at room temperature.
4. Aspirate the 4% paraformaldehyde and wash each well 3 times with 0.5 ml of PBS for 5 minutes with gentle agitation.
5. Add 0.5 ml per well of Blocking Buffer to block non-specific antibody binding.

Note: *The Blocking Buffer formulation depends on if the antigen is on the surface or is intracellular. Refer to the preparation section to make sure the appropriate Blocking Buffer is used.*

6. Incubate at room temperature for 1 hour.
7. Dilute the primary antibody in Blocking Buffer according to the manufacturer's instructions.
8. Aspirate the Blocking Buffer and add 200 µl of the diluted antibody to each well.
9. Incubate at 4°C overnight.
10. After the overnight incubation, wash each well 3 times with 0.5 ml of PBS for 10 minutes with gentle agitation.

Note: *If using a conjugated antibody, skip steps 11 through 14 and go directly to step 15. If using a purified primary antibody, continue to step 11.*

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11. Dilute an appropriate secondary antibody (fluorophore-conjugated) in Blocking Buffer according to the manufacturer's instructions.
12. Add 200 µl of diluted secondary antibody to each well.
13. Incubate at room temperature for one hour, protecting the plate from light.
14. Wash each well 3 times with 0.5 ml of PBS for 10 minutes with gentle agitation, protecting the plate from light.
15. For nuclear visualization during fluorescent imaging, prepare a 2 µg/ml working solution of DAPI by diluting the stock solution in PBS. Add to each well and incubate for 10 minutes at room temperature, protecting the plate from light.
16. Wash each well once with 0.5 ml of PBS for 5 minutes with gentle agitation, protecting the plate from light.
17. Aspirate any PBS remaining in the wells and, if desired, add 1 to 2 drops of mounting medium to each well to preserve the samples for fluorescence microscopy imaging.

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