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IMMUNOCYTOCHEMISTRY PROTOCOL

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details about the Immunocytochemistry.



Procedure

1h 30m

- 1 Select coverslips previously fixed in 4% paraformaldehyde and subsequently washed and stored in PBS solution.
- 2 **Permeabilize:** 0.2% TX-100 in PBS for  00:15:00 at  Room temperature . 15m

- 3 Wash 3 × 5 min in PBS. 5m

- 3.1 Wash  00:05:00 in PBS. (1/3) 5m

- 3.2 Wash  00:05:00 in PBS. (2/3) 5m

- 3.3 Wash  00:05:00 in PBS. (3/3) 5m

- 4 **Block:** 50% Sniper in PBS for  00:15:00 at  Room temperature . 15m

- 5 Wash 3 × 5 min in PBS. 5m

- 5.1 Wash  00:05:00 in PBS. (1/3) 5m

- 5.2 Wash  00:05:00 in PBS. (2/3) 5m

- 5.3 Wash  00:05:00 in PBS. (3/3) 5m




6 **Primary antibody:** mix desired antibody in PBS containing 5% sniper and 0.1% TX-100 overnight at  4 °C (gentle rocking).

7 Wash 3 × 5 min in PBS.



7.1 Wash  00:05:00 in PBS. (1/3)

5m



7.2 Wash  00:05:00 in PBS. (2/3)

5m



7.3 Wash  00:05:00 in PBS. (3/3)

5m



8 **Secondary antibody:** mix desired antibody in PBS containing 5% sniper and 0.1% TX-100.

- Can add DAPI in secondary solution or as a separate step following washing of the secondary.

9 Wash 3 × 5 min in PBS.



9.1 Wash  00:05:00 in PBS. (1/3)

5m



9.2 Wash  00:05:00 in PBS. (2/3)

5m



9.3 Wash  00:05:00 in PBS. (3/3)

5m



10 Mount coverslips to slide with Vectashield antifade mounting medium.