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IMMUNOCYTOCHEMISTRY PROTOCOL

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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 x 5 min in PBS.



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 x 5 min in PBS.



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 x 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 x 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.