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Technical Data Sheet

For research use only
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diagnostic or therapeutic use.*

Antibody Protocol For Frozen Tissue Sections

Immunohistochemistry Procedure for Staining Phosphoinositides (PIPs) in Frozen Tissue Sections

1. Slides are stored at -80 °C. Remove from freezer and air dry.
2. Fix the tissues for 20 minutes at -20 °C in cold acetone. Place directly into TBS.
3. Block in 10% Goat Serum (GS)-TBS for 30 minutes at 37 °C.
4. Rinse in TBS and blot the excess liquid from the slides. Place the primary antibody, at the dilution recommended in the data sheet, directly on the slides or place in a coplin jar and incubate for 30 minutes at 37 °C. The primary antibody can be saved and reused in a coplin jar approximately 3 times.
5. Wash in GS-TBS 2 times for 5 minutes at RT.
6. Add biotinylated secondary antibody from the AP Vector Kit (#AK-5010) for 30 minutes at 37 °C. Use 1 drop per 10 ml of GS-TBS.
7. Prepare the visualization system from the Vector kit. Use 2 drops A, mix, 2 drops B. **This is made 30 minutes in advance and left at RT until used.**
8. Wash in GS-TBS 2 times for 5 minutes at RT.
9. Incubate the slide with the visualization system from the Vector kit from step 7. Incubate for 30 minutes at 37 °C.
10. Wash in GS-TBS for 5 minutes.
11. Wash in TBS for 5 minutes.
12. Add vector red substrate (Vector SK-5100) for 30 minutes at RT in the dark.
13. Wash with TBS 2 times.
14. Counterstain with Hematoxylin for 10 minutes.
15. Wash 2 times with TBS.
16. Wash 2 times with nanopure or distilled H₂O.
17. Mount and view.

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