ProMab Biotechnologies, Inc.

Protocol

Immunohistochemistry (IHC)

OVERVIEW

Immunohistochemistry (IHC) is a wide-used biological technique that combines anatomy, physiology, immunology and biochemistry. Developed from the antigen-antibody binding reaction, immunohistochemistry can be considered as a method that visualize distribution and localization of specific antigen or cellular components in separated tissues, or tissue sections. Compared to other bio-techniques that are based on the antigen-antibody reaction such as immunoprecipitation, or western-blot, immunohistochemistry provides in situ information which promises a more convincing experimental result.

PROTOCOL

Prepare formalin-fixed, paraffin-embedded tissue sections (Step 1-8):

1. Fix freshly dissected tissue (<3mm thick) with 2% paraformaldehyde from 1h to overnight at room temperature.
2. Rinse the tissue with running tap water for 5 min.
3. Dehydrate the tissue through 70%, 80%, 95% alcohol, 5 min each, followed with 3 times of 100% alcohol, 5 min each.
4. Cleared the tissue in xylene for 2 times, 5 min each.
5. Immerse the tissue in paraffin for 3 times, 5 min each.
6. Embed the tissue in a paraffin block. The paraffin tissue block can be stored at room temperature for years.

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7. Section the paraffin-embedded tissue block at 5-8 µm thickness on a microtome and float in a 40°C water bath containing distilled water.

8. Transfer the sections onto glass slides suitable for immunohistochemistry. Allow the slides to dry overnight and store slides at room temperature until ready for use.

Immunostain formalin-fixed, paraffin-embedded tissue sections (Step 9-29):

9. Deparaffinize slides in xylene for 2 times, 5 min each.

10. Transfer slides to 100% alcohol, for 2 times, 3 min each, and then transfer once through 95%, 70% and 50% alcohols respectively for 3 min each.

11. Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol at room temperature for 10 min to block endogenous peroxidase activity.

12. Rinse with PBS for 2 times, 5 min each.

13. (Optional, recommended) Perform antigen retrieval to unmask the antigenic epitope. The most commonly used antigen retrieval is a citrate buffer method. Arrange the slides in a staining container. Pour 300 ml of 10 mM citrate buffer, pH 6.0 into the staining container and incubate it at 95-100°C for 10 min (optimal incubation time should be determined by user). Remove the staining container to room temperature and allow the slides to cool for 20 min.

14. Rinse slides with PBS for 2 times, 5 min each.

15. (Optional) Add 100 µl blocking buffer (e.g. 10% fetal bovine serum in PBS) onto the sections of the slides and incubate in a humidified chamber at room temperature for 1 h.

16. Drain off the blocking buffer from the slides.

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17. Apply 100 µl appropriately diluted primary antibody (in antibody dilution buffer, e.g. 0.5% bovine serum albumin in PBS) to the sections on the slides and incubate in a humidified chamber at room temperature for 1 h.
18. Wash the slides with PBS for 2 times, 5 min each.
19. Apply 100 µl appropriately diluted biotinylated secondary antibody (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min.
20. Wash slides with PBS for 2 times, 5 min each.
21. Apply 100 µl appropriately diluted Sav-HRP conjugates (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min (keep protected from light).
22. Wash slides with PBS for 2 times, 5 min each.
23. Apply 100 µl DAB substrate solution (freshly made just before use: 0.05% DAB - 0.015% H2O2 in PBS) to the sections on the slides to reveal the color of antibody staining. Allow the color development for < 5 min until the desired color intensity is reached. (Caution: DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.)
24. Wash slides with PBS for 3 times, 2 min each.
25. (Optional) Counterstain slides by immersing sides in Hematoxylin for 1-2 min.
26. Rinse the slides in running tap water for 10 min.
27. Dehydrate the tissue slides through 4 times of alcohol (95%, 95%, 100% and 100%), 5 min each.
28. Clear the tissue slides in 3 times of xylene and coverslip using mounting solution. The mounted slides can be stored at room temperature permanently.
29. Observe the color of the antibody staining in the tissue sections under microscopy.