Immunohistochemistry Protocol for Frozen Sections V.2

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Abstract

The following is a general procedure guide for preparation and staining of acetone-fixed **frozen** tissues using a purified, unconjugated primary antibody, biotinylated secondary antibody and streptavidin-horseradish peroxidase (Sav-HRP) and DAB detection system. Because each antigen differs in terms of requirement for fixation, amplification step, etc., it is not possible to write an inclusive protocol that will work for all antigens. The user must determine optimal conditions for each antigen of interest. Many protocols for staining individual antigens, as well as useful tips and troubleshooting guides for immunohistochemistry, can be found at the IHC World web site (http://www.ihcworld.com/).

Troubleshooting

Safety warnings

Caution: DAB is a suspected carcinogen. Handle with care. Wear gloves, lab coat andeye protection.

Before start

- For initial experiments, the user must titrate primary and secondary reagents so that staining with the secondary antibody alone yields no background while staining with primary and secondary antibodies yields strong, specific staining.
- Prepare the acetone fixative fresh and chill to -20°C.
- The protocol can be stopped after sectioning, at step 8, after which samples can be stored indefinitely in a slide box at -80°C.
- The protocol can also be paused at step 16, in which samples can be left to stain overnight at 4°C in the dark in a humidified chamber (cat no. 926301).
- If background is high with the secondary antibody, consider blocking with 2-10% normal serum from the host in which the secondary antibody was raised. I.e. if a goat anti-rabbit antibody secondary demonstrates high background, consider blocking with 2-10% normal goat serum at the Sample Blocking step.
- High background can also be mitigated by increasing the number and length of wash steps.
- Apply and aspirate buffers and solutions carefully so as not to detach the cells from their culture vessel, coverslip or slide.
- Take care to ensure that slides do not dry out by incubating with sufficient volumes and/or in a humidified chamber (cat no. **926301**).

Prepare frozen tissue sections

1 Place a freshly dissected tissue block (<5 mm thick) on to a pre-labeled tissue base mold.

2 Cover the entire tissue block with cryo-embedding media (e.g. OCT).

3 Slowly place the base mold containing the tissue block into liquid nitrogen till the entire tissue block is submerged into liquid nitrogen to ensure tissue is frozen completely.

4 Store the frozen tissue block at -80°C until ready for sectioning.

5 Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.

6 Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.

7 Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).

8 Dry the tissue sections overnight at room temperature. Sections can be stored in a sealed slide box at -80°C for later use.
16:00:00

Immunostain frozen tissue sections

9 Fix the tissue sections with a suitable fixative. One of the commonly used fixation methods for frozen tissue sections is to immerse the slides in pre-cooled acetone (-20°C) for 10 min.
00:10:00

10 Pour off the fixative and allow acetone to evaporate from the tissue sections for ≥ 20 min at room temperature.
00:20:00

11 Rinse the slides in 300ml of 10mM phosphate buffered saline (PBS) at a neutral pH for 2 changes, 5 min each.
00:05:00

12 Incubate the slides in 0.3% H_2O_2 solution in PBS at room temperature for 10 min to block endogenous peroxidase activity.
00:10:00

13 Rinse the slides in 300ml PBS for 2 changes, 5 min each.
00:05:00

14 (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 1h.
01:00:00

15 Drain off the blocking buffer from the slides.

16 Apply 100 µl an 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 for 1 h at room temperature or overnight at 4°C. 01:00:00

17 Rinse the slides in 300ml PBS for 2 changes, 5 min each.
00:05:00

18 Apply 100µl an 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.
00:30:00

19 Rinse the slides in 300ml PBS for 2 changes, 5 min each.
00:05:00

20 Add 100 µl pre-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).
00:30:00

21 Rinse the slides in 300ml PBS for 2 changes, 5 min each.
00:05:00

22 Apply 100 µl DAB substrate solution (freshly made just before use: 0.05% DAB - 0.015%H_2O_2 in PBS) to the sections on the slides to reveal the color of the antibody staining. Allow the color development for ≤ 5 min until the desired color intensity is reached.

Note

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23 Wash slides in 300ml PBS for 2 changes 5 min each.
00:05:00

24 (optional) Counterstain slides by immersing sides in Hematoxylin (e.g. Gill's Hematoxylin) for 1-2 min.
00:02:00

25 Rinse the slides in running tap water for ≥ 15 min.
00:15:00

26 Dehydrate the tissue slides through 4 changes of alcohol (95%, 95%, 100% and 100%), 5 min each.

27 Clear the tissue slides in 3 changes of xylene and coverslip using mounting solution (e.g. Permount). The mounted slides can be stored at room temperature permanently.

28 Observe the color of the antibody staining in the tissue sections under microscopy.