LP106 – IMMUNOHISTOCHEMISTRY STAINING

We describe below general recommendations for antibody staining of paraffin-embedded tissues with antigen retrieval protocols. Each antibody reacts differently with embedded tissues, therefore different protocols and conditions have to be tested to obtain the optimal results for end-user application.

Solution Preparation

PBS, pH 7.4: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 50 mM NaCl, 2.7 mM KCl
Blocking Buffer A: PBS, pH 7.4, 5% BSA, 0.5% Tween-20
Blocking Buffer B: PBS, pH 7.4, 5% BSA

Supplies

Vectastain ABC Kit (Cat. No. PK-6100), Vector Laboratories
DAB Substrate Kit for Peroxidase, (Cat. No. SK-4100), Vector Laboratories
Permount (histological mounting medium, Cat. No. SP15-100), Fisher Scientific

Protocol

1. Deparaffinize and rehydrate sections as follows: 3 x 3 min with xylene; 3 x 2 min with 100% ethanol; 2 min with 95% ethanol, 2 min with 80% ethanol, 2 min with 70% ethanol, and 5 min with PBS.
2. Retrieve antigen using one the antigen retrieval methods described below.
3. Block endogenous peroxidases by soaking slides in a solution of 90% methanol/3% H_2O_2 for 15 min at room temperature (RT). Then wash 3 x 5 min with PBS.
4. Shake and wipe off excess PBS. Circle all sections with a pap pen. Add 75 ul of Blocking Buffer A to each section immediately. Do not touch sections with tip.
5. Incubate 1 hr to overnight at RT in a humidified chamber. Do not let the slides touch each other.
6. Dilute primary antibody in Blocking Buffer A (dilutions will vary depending on tested antibody). Add 75 ul per section and incubate 1 hr to overnight at RT in a humidified chamber.
7. Drain primary antibody off section. Wash slides 3 x 10 min in PBS. You may have to wash slides in PBS + 0.1%-0.5% Tween-20 for some primary antibodies.
8. Dilute secondary antibody 1:1,000 in Blocking Buffer B. Add 75 ul per section and incubate for 1 hr at RT in a humidified chamber.
9. Drain secondary antibody and wash slides 5 x 10 min in PBS + 0.1% Tween-20. *(For secondary antibodies that are peroxidase conjugated, go to step 11)*.
10. Make ABC according to Vector protocol 30 min before time of use (mix 5 ml of PBS with 2 drops of solution A and 2 drops of solution B). Incubate samples for 45 min at RT. Wash 5 min in PBS.

11. Make DAB according to Vector protocol in ddH_2O. WEAR GLOVES: Mix 5 ml ddH_2O with 2 drops of buffer, 4 drops of DAB and 2 drops of H_2O_2. (if you want a gray-black stain, add 2 drops of the Nickel solution, and mix). Add immediately to slides and wait for color change (approximately 2-10 min). Drain slides and place into ddH_2O for 5 min. Dispose of DAB waste with bleach.

12. Counterstain with methyl green (1 min) or hematoxylin (3 sec). Wash 3 times with ddH_2O.
13. Immediately dehydrate in 70% ethanol, 80% ethanol, and 100% ethanol (one dip each).
14. Mount with Permount and seal coverslip with nail polish.

Antigen retrieval methods

- Sodium Citrate Antigen Retrieval:
  1. Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 total) to ensure even heating.
  2. Place rack in 600 ml of 10 mM sodium citrate, pH 6.0 in a glass 2L-beaker. Mark a line at the top of the liquid on the beaker.
  3. Microwave for 20 min total, replacing evaporated water every 5 min.
  4. Cool slides for 20 min.
  5. Wash 4 x 3 min in ddH_2O, and 3 min 1X PBS.

- Proteinase K Antigen Retrieval:
  1. Make a fresh solution of:
     25 ul of 20 mg/ml Proteinase K
     2.5 ml of 1 M Tris-CI, pH 8.0
     0.5 ml of 0.5 M EDTA, pH 8.0
     qs 50 ml with ddH_2O
  2. Incubate slides in a glass slide holder with solution at 37°C for 5 min (do not pre-warm Proteinase K solution).
  3. Wash 3 x 5 min with 1X PBS.

- Urea Antigen Retrieval:
  1. Make a fresh solution of 1 M urea.
  2. Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 total) to ensure even heating.
  3. Place rack in 600 ml of 1 M urea in a glass 2L-beaker. Mark a line at the top of the liquid on the beaker.
  4. Microwave for 10, 20 and 30 min total, replacing evaporated water every 5 min.
  5. Cool slides for 30 min to 1 hr.
  6. Wash 4 x 3 min in ddH_2O, and 3 min in 1 X PBS.