Immunohistochemistry of tissue slices

Solutions

Paraformaldehyde fixation solution (PFA)
4% paraformaldehyde
PBS (use TBS instead of PBS hereafter when an alkaline phosphatase is the marker enzyme)
Heat the solution (≤70°C) to dissolve paraformaldehyde. Note: Paraformaldehyde is hazardous!

Blocking solution
Goat Serum (GS) and Gelatin (Gel), for concentrations see procedure (*Note: Gel is not required*)
1-3% Bovine Serum Albumin (*Note: Not required*)
0.1-1% Triton X100
0.05% Sodium Azide (NaN_3, Sigma S-2002)
PBS

Antibody (Ab) solutions
Ab-solutions with desired concentrations of primary and secondary Ab are mixed in blocking solution (+ 1% GS + 0.04% Gel). Add 250 µl Ab-solution per well in a 24 well cassette.

Procedure

After the secondary Ab is applied, all steps must be done in dark (or covered with aluminum foil). Unspecific fluorescence can be reduced if the blocking solution is filtered using a syringe driven filter unit 0.22 µm. Prepare a coverslip with only the secondary Ab to check for unspecific immuno staining. To remove all aldehyde traces, incubate slices in freshly made sodium borohydride solution (0.5% sodium borohydride in PBS) after PFA fixation in step 2. The slices can be post-fixed (after step 9) in PFA for 15 min followed by washing in PBS 3x15 min.

1. After sectioning, rinse slices in PBS for 10 min at room temperature (RT).
2. Fix slices in ice-cold PFA for 1 h at 4°C.
3. Incubate with blocking solution (+ 10% GS + 0.2% Gel) for 1 h to overnight, shaking at 4°C.
4. Wash in blocking solution (+ 1% GS + 0.04% Gel) for 1 h, shaking at 4°C.
5. Incubate with primary Ab-solution overnight to 3 days, shaking at 4°C.
6. Wash in blocking solution (+ 1% GS + 0.04% Gel) 3 times for 15 min, shaking at 4°C.
7. Incubate with secondary Ab-solution for 1 h to overnight, shaking at 4°C.
8. Wash in blocking solution (+ 1% GS + 0.04% Gel) 2 times for 15 min, shaking at 4°C.
9. Wash with clean PBS 3 times for 15 min, shaking at 4°C.
10. Mount the coverslips using Prolong Antifade Kit (Molecular Probes No. P-7481).
11. Leave the slides in dark RT overnight and examine slides the next day. Store slides in -20°C.

Wet the surface of coverslips with a paintbrush and transfer slices onto the coverslip using the paintbrush or a cut plastic Pasteur pipette. Make sure that the slices are fully spread. Remove access medium and let slices get nearly dry. Put mounting medium onto the glass slide and carefully drop the coverslip on the mounting medium. To make the tissue perfectly flat apply a small weight (e.g. a nut or bolt) on the coverslips when the slides are drying overnight.