IHC Protocol (paraffin)
Mouse primary antibody

Solutions and reagents

Lysis buffers

10 x Citrate Buffer:

- 29.4 g Tris-Sodium Citrate 2-hydrate ($C_6H_5Na_3O_72H_2O$)(= 0.1 M)

1. Resolve in 800 ml ddH_2O.
2. Adjust pH to 6.0 using citric acid.
3. Fill it up to 1 l.
4. Store at 4°C.
5. Dilute 1:10 before use.

10 x PBS:

- 80 g NaCl
- 2 g KCl
- 14 g Na_2HPO_4
- 2.4 g $KH_2PO_4$15O mM NaCl
- 1.0% NP-40 (or 0.1% Triton X-100)
- protease inhibitors

1. Resolve in 800 ml ddH_2O.
2. Adjust pH to 7.4 using HCl.
3. Fill it up to 1 l.
4. Autoclave it.
5. Store at RT.
6. Dilute 1:10 before use.

Procedure

Deparaffinization

- Xylol 5 min
- Xylol 5 min
- 100% EtOH 3 min
- 100% EtOH 3min
- 3% H_2O_2 in 70% EtOH 10 min
- ddH_2O 1min

1. Antigen retrieval for 30 min in 10 mM Citrate buffer (preheated; start the steam cooker 10 min in advance; use plastic cuvettes for the slides).
2. Let the slides cool down in the buffer to RT (for approximately 40 min).
3. Wash the slides 2x 5 min in 1x PBS (shaking).
4. Encircle the sections with DAKO-Pen during the washing time in 1x PBS.
5. Prepare a moist chamber for the staining procedure.

Staining

1. Incubate the sections with normal serum (VectorLaboratories, MP-7402: ImmPRESS® HRP Anti-Mouse IgG (Peroxidase) Polymer Detection Kit) for 20 min at RT.
2. Remove the normal serum from the sections (knocking off, do not wash!).
3. Dilute the primary antibody with 1x PBS and apply it to the sections.
4. Incubate the primary antibody ON at 4°C or 60 min at RT in a wet chamber.
5. Remove the primary antibody from the slides (knocking off) and wash 2x 5 min in 1x PBS (shaking).
6. Incubate the sections with the secondary antibody (VectorLaboratories, MP-7402: ImmPRESS® HRP Anti-Mouse IgG (Peroxidase) Polymer Detection Kit) for 20 min at RT in a wet chamber.
7. Wash the slides 2x 5 min in 1x PBS (shaking).
8. Prepare the DAB solution (VectorLaborarories, SK-4100) according to manufacturer's recommendations briefly before use and mix it well.
9. Incubate slides with DAB solution until a brown staining is visible. The development time is varying! (from a few seconds to a few minutes; watch closely)
10. Put the slides 3 min in 50 mM $NaHCO_3$.
11. Wash the slides briefly in ddH_2O.
12. Put the slides in Haemalaun (the time is varying-from a few seconds to a few minutes).
13. Wash the slides under rinsing tap water for 10 min.

Alcohol series and Xylol

- 70% EtOH briefly
- 96% EtOH briefly
- 100% EtOH briefly
- 100% EtOH 2 min
- Xylol briefly
- Xylol 2 min

Cover the sections with Eukitt and cover slip.