IHC Protocol (paraffin)

guinea pig primary antibody

Solutions and reagents:

10x Citrat buffer:

29.4 g Tri-Sodium Citrate 2-hydrate ($C_6H_5Na_3O_72H_20$)(= 0.1 M)

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

10x PBS:

80 g NaCl
2 g KCl
14 g Na_2HPO_4
2.4 g KH_2PO_4

- Resolve in 800 ml ddH_2O.
- Adjust pH to 7.4 using HCl.
- Fill it up to 1l.
- Autoclave it.
- Store at room temperature.
- Dilute 1:10 before use.

Procedure

Deparaffinization:

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

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

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Staining:

- Incubate the sections with 10% serum (of the species the secondary antibody is generated in) in 1% BSA in PBS for 20 minutes at room temperature.
- Remove the normal serum from the sections (knocking off, do not wash!).
- Dilute the primary antibody with 1x PBS and apply it to the sections.
- Incubate the primary antibody ON at 4°C or 60 minutes at RT in wet chamber.
- Remove the primary antibody from the slides (knocking off) and wash 2 x 5 minutes in 1x PBS (shaking).
- Incubate the sections with the secondary antibody (anti-guinea pig Biotin conjugated) for 30 minutes at RT in wet chamber.
- Prepare ABC solution of ABC-Standard kit (VectorLaboratories, PK-4000) according to manufactures recommendations, incubate the solution for 30 min.
- Wash the slides 2 x 5 minutes in 1x PBS.
- Apply ABC-standard to the slides, inbubate 30 min at RT in wet chamber.
- Wash the slides 2 x 5 minutes in 1x PBS.
- DAB (VectorLaborarories, SK-4100):

5ml	ddH_2O
2 drops	buffer
4 drops	DAB
2 drops	hydrogen peroxide solution

  Prepare the DAB briefly before use and mix it well.
- The development time is varying! (from a few seconds to a few minutes; watch closely)
- Put the slides 3 minutes in 50mM $NaHCO_3$.
- Wash the slides briefly in ddH_2O.
- Put the slides in Haemalaun (the time is varying-from a few seconds to a few minutes).
- Wash the slides under rinsing tap water for 10 minutes.

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.

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