IHC Protocol (paraffin)
- rabbit primary antibody -

Solutions and reagents

10x Citrat buffer:

29.4 g Tri-Sodium Citrate 2-hydrate ($C_6H_5Na_3O_7 2H_2O$)(= 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

1. Antigen retrieval for 30 minutes in 10mM 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 room temperature (for approximately 40 minutes).
3. Wash the slides 2x 5 minutes 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.

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Staining

1. Incubate the sections with normal serum (PROGEN, prohisto-HiSec normal goat serum (ready-to-use), prohisto-HiSec anti-rabbit HRP detection kit, Cat. No. PRSTAR) for 20 minutes at room temperature.
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 minutes at room temperature.
5. Remove the primary antibody from the slides (knocking off) and wash 2 x 5 minutes in 1x PBS (shaking).
6. Incubate the sections with the secondary antibody (PROGEN, prohisto-HiSec anti-rabbit HRP (ready-to-use, prohisto-HiSec anti-rabbit HRP detection kit, Cat. No. PRSTAR) for 20 minutes at room temperature.

1. DAB (prohisto-HiVis DAB duo, Cat. No. PRDAB1):

   Reagent A & Reagent B
   mix 1:1

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

Alcohol series and Xylol

1.	70% EtOH	briefly
2.	96% EtOH	briefly
3.	100% EtOH	briefly
4.	100%EtOH	2 min
5.	Xylol	briefly
6.	Xylol	2 min.

Cover the sections with Eukitt and cover slip.

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