

IHC Protocol (paraffin)

rabbit primary antibody

Solutions and reagents:

10x Citrat buffer:

29.4 g Tri-Sodium Citrate 2-hydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) (= 0.1 M)

- Resolve in 800 ml ddH₂O.
- 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 Na₂HPO₄
2.4 g KH₂PO₄

- Resolve in 800 ml ddH₂O.
- 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% H₂O₂ in 70% EtOH 10 min
- ddH₂O 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.

