

# IHC Protocol (paraffin)

## mouse 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 ddH<sub>2</sub>O.
- 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<sub>2</sub>HPO<sub>4</sub>  
2.4 g KH<sub>2</sub>PO<sub>4</sub>

- Resolve in 800 ml ddH<sub>2</sub>O.
- 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<sub>2</sub>O<sub>2</sub> in 70% EtOH 10 min
- ddH<sub>2</sub>O 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.

