

## **Protocol Immunohistochemistry for anti-human Prox-1** (Cat# 102-PA32, RELIA Tech GmbH)

(AG. Funct. Anatomy: Prof. Gasse / PD Dr. C. Staszkyk)

### 1. Day:

- 2 x 5min Xylol
- 5min Isopropanol
- 5min 96% EtOH
- 5min 70% EtOH
- 5min 50% EtOH
- 5min 20% EtOH
- Aqua dest

- put object slide for 20min into boiling Citrate buffer (ph 6) within a boiling water bath
- cool down for about 20 min
- clamping into cover plates
- rinse 3 x 5min with Tris buffer
- 10min Pepsin at 37°C (je 200 µl)

[Pro Taqs Pepsin Digest (Acetic) Quartett, Immundiagnostika und Biotechnologie GmbH, Berlin]

- rinse 3 x 5min with Tris buffer
- 10min Peroxidase-Block (10% H<sub>2</sub>O<sub>2</sub> in MeOH)
- rinse 3 x 5min with Tris buffer
- 30min normal goat serum

[Proteinblock, Normal goat serum, BioGenex (each 2Tr.)]

- primary antibody – over night at 4°C
- [antibody dilution: 1:400 (in Aqua bidest)]

### 2. Day:

- rinse 3 x 5min with Tris buffer
- incubate 20min with secondary bridge-antibody
- [Super Sensitive Link-Label ICH Detection System, BioGenex]
- rinse 3 x 5min with Tris buffer
- incubate 20min with Streptavidin – Peroxidase – Label

[Super Sensitive Link-Label ICH Detection System, BioGenex]

- rinse 3 x 5min with Tris buffer
- incubate 5min with DAB
- [Liquid DAB , BioGenex]
- Aqua dest

- 10sec Toluidin blue; Aqua dest; 80 % EtOH; ascending row – cover with DePeX
- [DePeX: Serva 18243]

### Solutions:

Tris buffer: 6,1g Trizma Base, 37ml 1N HCl, 8g NaCl, 0,1ml Tween 20 (0,01%)  
ad 1000ml Aqua dest, pH 7,6 (+/-0,2)

Citrate buffer: Stock solution A: 0,1M citric acid  
Stock solution B: 0,1M sodium citrate  
Working solution: 18ml solution A + 82ml solution B  
ad 1000ml Aqua dest; pH 6