

Immunohistochemistry for EM: Pre-embedding immunoperoxidase

- 1) Sections were washed in TBS (30 min).
- 2) Blocking solution: 10% Normal Goat Serum (NGS) in TBS (1 h.).
- 3) Primary antibodies (final protein concentration of 0.5-2 μ g/ml in TBS with 2% NGS (overnight at 4°C.).
- 4) Wash in TBS (3X15 min.).
- 5) Biotinylated secondary antibodies in TBS containing 2% NGS (2h.).
- 6) Wash in TBS (2x15min.).
- 7) ABC complex in TBS (75 or 90 min.).
- 8) Wash in TBS (2x15 min.).
- 9) Wash in TB (2x15 min.).
- 10) Incubate sections in 3-3'-diaminobenzidine tetrahydrochloride (DAB) 0.05% in TB, pH 7.4, (5 min.).
- 11) Add 0.01% H₂O₂ in distilled water. Develop the reaction for several minutes.
- 12) Stop the reaction in TB
- 13) Wash sections in TB (4x10 min.).
- 14) Wash sections in 0.1M PB (15min.).
- 15) Osmication: 1% osmium tetroxide in 0.1M PB (30 min.).
- 16) Wash sections in 0.1M PB (5x8 min.).
- 17) Wash sections in distilled water (5min.)
- 18) Uranyl acetate: 1% in distilled water (30min.).
- 19) Dehydration: 50%,70%, 90%, 96%, 100% and 100% (10 min. each).
- 20) Propylene oxide (2x10 min.).
- 21) Embedding in resin.
- 22) Polymerization in oven at 65°C.