



### Antigen Retrieval of Rat Brain Sections

- 1) Wash sections four times with ice-cold 100 mM PB (100 mM Na Phosphate pH 7.4) with gentle rocking/agitation at 4°C for 5 min each wash.
- 2) Antigen retrieval
  - Option 1: Citrate method:
    - a) Wash sections with 10 mM Sodium citrate pH 8.5 with gentle rocking/agitation at room temperature (RT) for 5 min.
    - b) Incubate sections in 10 mM Sodium citrate pH 8.5 with gentle rocking/agitation at 80°C for 30 min.
  - Option 2: Pepsin method:
    - a) Wash sections in purified H<sub>2</sub>O with gentle rocking/agitation at 37° C for 5 min.
    - b) Incubate sections in 0.15 mg/mL Pepsin dissolved in 0.2N HCl with gentle rocking/agitation at 37°C for 10 min.
- 3) Cool sections to RT.
- 4) Wash sections two times with ice-cold 100 mM PB.
- 5) Proceed to the blocking step of the antibody labeling protocol.

#### References:

Citrate: Jiao et al 1999 *J Neurosci Methods* (<http://www.ncbi.nlm.nih.gov/pubmed/10634500>)

Pepsin: Fukaya & Watanabe 2000 *J Comp Neurol* (<http://www.ncbi.nlm.nih.gov/pubmed/11027400>)