

## **Acid phosphatase enzyme histochemistry in mouse brain**

- i. Mice are transcardially perfused with 4% PFA, 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) containing 0.025% calcium chloride, 5% sucrose and 0.075% cytidine 5'-monophosphate (CMP).
- ii. The brains are removed and further immersion-fixed in 4% PFA for 4 h at 4°C.
- iii. Vibratome sections (50- $\mu$ m) are cut, rinsed thoroughly in 0.1 M sodium cacodylate buffer containing 5% sucrose, then rinsed twice in 0.05 M Tris-maleate buffer, pH 5, containing 5% sucrose (see below).
- iv. Sections are incubated in the reaction medium [25 mg CMP, 7 ml distilled water, 10 ml 0.05 M Tris-maleate buffer with 5% sucrose, 5 ml 0.025 M manganese chloride, 2.4~3 ml 1% lead nitrate (prepared drop by drop while stirring, so as not to have any white precipitate after lead nitrate addition), pH 5.0, filtered with #50 paper) for 1 h at 37°C.
- v. After washing twice in Tris-maleate buffer and twice in 0.1 M sodium cacodylate buffer containing 5% sucrose, sections are briefly treated with 1% sodium sulfide in 0.1 M sodium cacodylate buffer containing 5% sucrose, and rinsed well in sodium cacodylate buffer containing 5% sucrose. The sections are then post-fixed in 1% osmium tetroxide for 30 min and processed for EM embedding.

---- Tris(hydroxymethyl)aminomethane-maleate (Tris-maleate) buffer, 0.05 M with 5% Sucrose:

For a total of 200 ml:

- Add Tris(hydroxymethyl)aminomethane-maleate 1.21 g to 150 ml dH<sub>2</sub>O, stir;
- Add maleic acid 1.16 g, stir;
- Add 6 ml pre-made 0.2 M NaOH – this will lead to a pH of 5, stir;
- Add sucrose 10 g, stir;
- Add dH<sub>2</sub>O to have a final 200 ml.