Electron microscopy on culture cells

1) Fix cultures with 4% paraformaldehyde + 0,1% glutaraldehyde + 15% saturated solution of picric acid in 0.1M PB (ph 7.4) (freshly prepared) for 2 hours.

2) Wash cultures in 0.1M PB (ph 7.4) for 2 hour (eight times for 15 min each wash).

3) Wash in 0.1 M PB.

4) Postfix cultures in 1% OsO4 in 0.1 M PB (for 30 min. on the dark).

5) Wash in 0.1 M PB (5 x 5 min.).

6) Wash with distilled water (for 5 min.).

7) 1% Uranyl acetate in distilled water (for 30 min. on the dark).

8) Dehydration (50°, 70°, 90°, 95°, 100° I, 100° II ethanol) (for 10 min. each one).

9) Propilene oxide (2 x 10 min.).

10) Durcupan resin (20 g A; 20 g B; 0.6 g D; 0.6 g C) (3 hours).

11) The cultures will be embedded in resin.

Buffers and fixative

- Buffer: 0.2M sodium phosphate buffer (PB), pH 7.4. A mixture of 35.6 g/L of $Na_2HPO_4 \bullet 2H_2O$ and 31.2 g/L of $NaH_2PO_4 \bullet 2H_2O$, each at 0.2 *M*, in the ratio 4:1 (v/v), has a pH of approx 7.4. To prepare 0.1M PB, mix the same proportion of 0.2M PB with distilled water.

- To prepare 1000 mL of 4% paraformaldehyde + 0.1% glutaraldehyde + 15% saturated solution picrid acid in 0.1 *M* PB, pH 7.4: dissolve 40 g of paraformaldehyde in 250 mL of distilled water, add 150 mL of saturated solution picrid acid, make up to 500 mL with distilled water, filter, add 500 mL of 0.2 *M* PB, and add 4 mL of 25% glutaraldehyde. For safety reasons, all these steps should be carried out in a fume hood.