

Immunoelectron microscopy with nano-gold particles and silver enhancement

1- Grow adherent cells on glass coverslips (ideally, pre-treated with poly-L-lysine to preserve the mitotics) under normal growing conditions. Scribe the desired cell with a diamond scribe and take snapshots at low magnification of the cell and scribe.

2- Remove the medium and wash the cells once with PHEM (60 mM PIPES, pH 6.9, 25 mM HEPES, 10mM EGTA, 2 mM MgCl₂).

3- Permeabilize the cells by incubation in 0.2% Triton X-100 in PHEM for 1 min and fix immediately with 0.5% glutaraldehyde in PHEM for 5 min at RT. (**NOTE:** Glutaraldehyde causes green auto-fluorescence and any attempt to scribe, for example, a GFP-transfected cell post-fixation will result in heavy frustration)

4- Remove fixative and add 0.5 mg/ml of freshly made NaBH₄ (stored in a desiccator) in dulbecco's PBS for 5 min to reduce free aldehydes. Wash 3 times with dulbecco's PBS.

5- Block with KB⁻ (10 mM TRIS HCl pH 7.7, 150 mM NaCl) supplemented with 5% BSA for 30 min at RT.

6- Wash 2x 5 min with INCUBATION BUFFER (KB⁻ supplemented with 0.1% AURION BSA-c; AURION, Netherlands). (**NOTE:** AURION BSA-c is an acetylated and partly linearized form of BSA that improves signal-to-noise ratio and SHOULD NOT be used as a blocking reagent, but ONLY during washes and antibody incubations).

6- Dilute your primary antibody (in the case of anti-GFP we used 1:100 dilution of ABCAM's antibody) in INCUBATION BUFFER and incubate for 1 h at RT.

7- Wash with INCUBATION BUFFER for 3x 5 min.

8- Incubate with 0.8 nm secondary goat anti-rabbit gold (AURION, Netherlands) diluted 1:100 in INCUBATION BUFFER for at least 4 hrs at RT (we can leave this overnight at 4 °C if required).

9- Wash 4x 5 min with INCUBATION BUFFER and than wash 2x 5 min with PHEM.

10- Post-fix with 2.5% glutaraldehyde in PHEM for 30 min and then wash 3x 5 min with PHEM. (**NOTE:** usually we leave the cells at this point overnight at RT in PHEM)

11- In the next morning, proceed with silver enhancement using AURION R-GENT SE-EM (cat #: 500.033; AURION, Netherlands).