Pre-embedding immunogold technique at the EM level

1) Perfuse-fix the animal, cut brain in a vibratome (60 µm-thick sections) and store sections in 0.1 M PB (pH 7.4) at 4ºC.
2) Wash sections in TBS (30 min).
3) Blocking solution: 10% Normal Goat Serum (NGS) in TBS (1 hour).
4) Primary antibodies (final protein concentration of 2-5 µg/ml) diluted in TBS with 2% NGS (overnight at 4ºC).
5) Wash sections in TBS (3 x 15 min).
6) Secondary antibodies: 1.4 nm-conjugated IgG (1:100 dilution) in TBS with 2% NGS (2 hours, RT).
7) Wash sections in TBS (2 x 15 min).
8) Wash sections in PBS (2 x 15 min).
9) Postfix sections in 1% glutaraldehyde in PBS (10 min).
10) Wash sections in PBS (2 x 10 min).
11) Wash sections in distilled water (2 x 10 min).
12) Silver enhancement (HQ Silver kit, Nanoprobes). Add one drop of A, one drop of B, and mix; then, add one drop of C, and mix. Incubation in darkness.
13) Stop reaction in distilled water.
14) Wash sections in distilled water (4 x 10 min).
15) Wash sections in 0.1M PB (15 min).
16) Osmication: 1% osmium tetroxide in 0.1M PB (30 min).
17) Wash sections in 0.1M PB (5 x 8 min).
18) Wash sections in distilled water (5 min)
19) Uranyl acetate: 1% in distilled water (30 min).
20) Dehydration: 50%, 70%, 90%, 96%, 100% I and 100% II (10 min each).
21) Propylene oxide (2 x 10 min).
21) Flat-embedding in epoxy resin (Durcupan) (10 g A + 10 g B + 0.3 g D + 0.3 g C).
22) Polymerization in oven at 55-60ºC.

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