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Procedure for the immunolabelling of sections with Guinea pig antisera

Material

- Free-floating cryostat or vibratome sections (fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) or paraffin sections of tissue fixed with Bouin's fluid or 10% formalin.
- 0.1 M Tris-buffered saline (TBS) pH 7.3.
- **SWant guinea-pig antiserum.**
- Biotinylated anti-guinea pig IgG.
- Avidin-peroxidase (or streptavidin-fluorescence dye [e.g. Cy2 or Alexa 488]) Peroxidase substrate (e.g. Diaminobenzidine (HCl) and H_2O_2 for immunoperoxidase).
- Ethanol and Xylol (only for immunoperoxidase).
- Mounting medium (e.g. Eukitt for immunhistochemistry, Hydromount for immunofluorescence).

Method

1. Apply the SWant guinea pig-antiserum diluted 1:1'000-1:5'000 (paraffin sections) or 1:5'000-1:10'000 (floating sections) in TBS with 10% carrier serum (e.g. calf or horse serum) and eventually 0.2% Triton-X 100 (particularly for vibratome sections). Incubate for 1 to 3 days at 4°C (on a shaker for free-floating sections, in a humid chamber for paraffin sections).
2. Rinse in TBS 3 x 5 min.
3. Apply biotinylated anti-guinea pig IgG-biotin (diluted according to the suggestions of the supplier) in TBS with 10% carrier serum. Incubate at room temperature (RT) for 1 to 4 hours.
4. Rinse in TBS 3 x 5 min.
5. Apply the avidin-biotin-peroxidase complex (diluted according to the suggestions of the supplier) or the Streptavidine-fluorescent complex in TBS with 10% carrier serum. Incubate for 1 to 2 hours at RT.
6. Rinse in TBS 3 x 5 min.
7. For immunoperoxidase: treat the section with a peroxidase substrate (e.g. Diaminobenzidine-HCl / H_2O_2).

8. Rinse in TBS 3 x 5 min.
9. Mount free floating sections on slides, eventually counterstain with cresyl-violet.
10. For immunoperoxidase-staining dehydrate with ethanol and xylol and mount and coverslip in Entellan. For immunofluorescence-staining mount and coverslip in Hydromount.

Note: in case of excessive background-staining, use higher dilutions of the SWant guinea pig antiserum.

*** Without prior fixation the highly soluble Ca2+-binding proteins are immediately lost during sectioning.**