Procedure for the immunolabelling of sections with rabbit antisera

Material

- Free-floating cryostate or vibratome sections (40-80 µm) of fixed tissue* (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) or paraffin sections (3-5 µm) of tissue fixed with 10% unbuffered formalin.
- 0.1 M Tris-buffered saline (TBS) pH 7.3.
- **SWant rabbit antiserum.**
- Biotinylated anti-rabbit IgG.
- Streptavidin-peroxidase or Streptavidin conjugated to CY2, Cy3, Cy5, or other fluorescent dyes.
- Peroxidase substrate (e.g. Diaminobenzidine(HCl) and H_2O_2).
- Ethanol and Xylol.
- Mounting medium (e.g. Eukitt for immunhistochemistry, Hydromount for immunofluorescence).

Method

1. Apply the SWant rabbit-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-rabbit 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 streptavidine-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. The immunofluorescent labelling is terminated at this point and the section can be mounted on slides and coverslipped with an aqueous medium (e.g. Hydromount).
8. The immunohistochemical reaction is concluded by treating the section with a peroxidase substrate (e.g. Diaminobenzidine HCl / H_2O_2) under continuous microscopic observation.
9. Rinse in TBS 3 x 5 min.

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10. Mount free floating sections on slides, eventually counterstain with cresyl-violet.
11. Dehydrate immunohistochemically treated sections with ethanol and xylol. Add mounting medium (Eukitt) and coverslip.

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

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

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