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## Procedure for the immunolabelling of sections with rabbit 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 or 10% unbuffered formalin.
- 0.1M Tris-buffered saline (TBS) pH 7.3.
- SWant rabbit antiserum.
- Biotinylated anti-rabbit IgG.
- Avidin-peroxidase.
- Peroxidase substrate (e.g. Diaminobenzidine(HCl) and H<sub>2</sub>O<sub>2</sub>).
- Ethanol and Xylol.
- Mounting medium.

### Method:

1. Apply the SWant 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 0.4% 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 10 min.
3. Apply 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 10 min.
5. Apply the avidin-biotin-peroxidase complex (diluted according to the suggestions of the supplier) in TBS with 10% carrier serum. Incubate for 1 to 2 hours at RT.
6. Rinse in TBS 3 x 10 min.
7. Treat the section with a peroxidase substrate (e.g. Diaminobenzidine HCl/ H<sub>2</sub>O<sub>2</sub>).
8. Rinse in TBS 3 x 10 min.
9. Mount free floating sections on slides, eventually counterstain with cresyl-violet.
10. Dehydrate with ethanol, xylol. Add mounting medium and coverslip.

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



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## Procedure for the immunolabelling of sections with goat 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 or 10% unbuffered formalin.
- 0.1M Tris-buffered saline (TBS) pH 7.3.
- SWant goat-antiserum.
- Biotinylated anti-goat IgG.
- Avidin-peroxidase.
- Peroxidase substrate (e.g. Diaminobenzidine(HCl) and H<sub>2</sub>O<sub>2</sub>).
- Ethanol and Xylol.
- Mounting medium.

### Method:

1. 1. Apply the SWant 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 0.4% 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 10 min.
3. Apply anti-goat IgG-biotin (diluted according to the suggestions of the supplier) in TBS
4. With 10% carrier serum. Incubate at room temperature (RT) for 2 to 4 hours.
5. Rinse in TBS 3 x 10 min.
6. Apply the avidin-biotin-peroxidase complex (diluted according to the suggestions of the supplier) in TBS with 10% carrier serum. Incubate for 1 to 2 hours at RT.
7. Rinse in TBS 3 x 10 min.
8. Treat the section with a peroxidase substrate (e.g. Diaminobenzidine HCl/ H<sub>2</sub>O<sub>2</sub>).
9. Rinse in TBS 3 x 10 min.
10. Mount free floating sections on slides, eventually counterstain with cresyl-violet.
11. Dehydrate with ethanol, xylol. Add mounting medium and coverslip.

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



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## Procedure for the immunolabelling of sections with mouse monoclonal antibodies

### 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 or 10% unbuffered formalin.
- 0.1M Tris-buffered saline (TBS) pH 7.3.
- SWant monoclonal antibody.
- Biotinylated anti-mouse IgG.
- Avidin-peroxidase.
- Peroxidase substrate (e.g. Diaminobenzidine(HCl) and H<sub>2</sub>O<sub>2</sub>).
- Ethanol and Xylol.
- Mounting medium.

### Method:

1. Apply the SWant antibody 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 0.4% 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 10 min.
3. Apply anti-mouse IgG-biotin (diluted according to the suggestions of the supplier) in TBS
4. With 10% carrier serum. Incubate at room temperature (RT) for 2 to 4 hours.
5. Rinse in TBS 3 x 10 min.
6. Apply the avidin-biotin-peroxidase complex (diluted according to the suggestions of the supplier) in TBS with 10% carrier serum. Incubate for 1 to 2 hours at RT.
7. Rinse in TBS 3 x 10 min.
8. Treat the section with a peroxidase substrate (e.g. Diaminobenzidine HCl/ H<sub>2</sub>O<sub>2</sub>).
9. Rinse in TBS 3 x 10 min.
10. Mount free floating sections on slides, eventually counterstain with cresyl-violet.
11. Dehydrate with ethanol, xylol. Add mounting medium and coverslip.

Note : in case of excessive background-staining use higher dilutions of the SWant monoclonal antibody.