Immunohystochemical staining, vibratome sections

Floating sections in 24-well plate in 1x PBS

- **1 x PBS 3% H_2O_2** 10 min RT on the wobbler (to quench endogenous peroxidase activity); 500 µl / well.
  - 1 x PBS 0.1% TritonX100 rinse
  - 1 x PBS 0.1% TritonX100 5 min on wobbler
  - 1 x PBS 0.1% TritonX100 5 min on wobbler

- **250 µl Primary Abs** (in 1 x PBS 0.1% TritonX100 + 10% serum (accordingly to the secondary Abs) Overnight RT on wobbler
  - 1 x PBS 0.1% TritonX100 rinse
  - 1 x PBS 0.1% TritonX100 5 min on wobbler
  - 1 x PBS 0.1% TritonX100 5 min on wobbler

- **250 µl biotinylated Secondary Abs** (in 1 x PBS 0.1% TritonX100) 30 min RT on wobbler
  - 1 x PBS 0.1% TritonX100 rinse
  - 1 x PBS 0.1% TritonX100 5 min on wobbler
  - 1 x PBS 0.1% TritonX100 5 min on wobbler

- **250 µl Str-Hrp 1/1000** (in 1 x PBS 0.1% TritonX100) 30 min RT on wobbler
  - 1 x PBS 0.1% TritonX100 rinse
  - 1 x PBS 0.1% TritonX100 5 min on wobbler
  - 1 x PBS 0.1% TritonX100 5 min on wobbler

- **250 µl DAB + H_2O_2** (1,4 µl H_2O_2 for 5 ml filtered DAB solution) – *DAB solution: 10 mg DAB (=1 tablet) for 25 ml 0.05 TRIS (TBS) pH 7,6; dissolve and filter through 0.22µM filter,* add H_2O_2 just before use! Or Vector SG (TH)
  Allow reaction to **proceed** for a few minutes, RT without wobbling
  - 1 x PBS 0.1% TritonX100 rinse
  - Replace PBS TritonX100 with PBS or PBS + 0,1% Na Azide in case sections are not to be mounted immediately. Store at +4°C.

- Briefly **rinse sections in ½ PBS + ½ AD** and allow to dry for about 30 min in flow.

- **Dehydratation:**
  - 5 min 70% ethanol
  - 5 min 90% ethanol
  - 5 min 100% ethanol
  - 5 min 100% ethanol
  - 5 min Histoclear II

- **Mount** coverslips on top of the slides with DPX and allow to dry overnight in the flow. Press out bubbles next morning.

1st AB: TH Ab152 1/5000
2nd AB: SAR 1/300
STRP-HRP 1/1000