Immunohistochemistry on paraffin embedded sections

Many labs use different methods, and we get IHC data from different sources using different protocol. We advise diluting the primary antibody in blocking buffer (PBST or TBST with BSA). Below is a summary of the most important steps. Some variations may apply.

- Remove the paraffin by several changes of xylene (5 min each at ambient temp).
- Rehydrate the sections gradually going from 3x 100% , 2x 95%, 1x 80%, 1x 50% alcohol, 3min each at ambient temp.
- Rinse in gently running water, 5 min ambient temp.

Antigen Retrieval

Generally sections are exposed to heat at either pH6 or pH9. Some labs use a microwave, others use a steaming bath. In our opinion best results are obtained using a high pressure pan especially designed for this purpose with precision controlled temp and pressure. In this case half of the slides are submerged in a buffer at pH6 (10mM sodium citrate) and the other half of the slides in a buffer at pH9 (10mM Tris/EDTA).

- Leave the slides to gently cool down to ambient temp.

For HRP staining

- Rinse the slides in water
- Block endogenous peroxidise activity by 20% H2O2 (15min) or commercial blocking agent (DAKO Cytomation for 5min).
- Wash in PBS 2x 5min
- Follow labelling protocol for primary and secondary antibodies (do not forget to block and wash in between)
- Add DAB for 5 min.
- Wash with water frequently for at least 15min
- Counterstain with haematoxylin (optional)
- Wash in tap water for at least 15 min and mount with aquamount

For AP staining

- Rinse the slides in TBS with 0.05%Tween (TBST), 1 min.
- Follow labelling protocol for primary and secondary antibodies (do not forget to block and wash in between)
- Wash in PBS 2x 5min
- When the secondary antibody was biotinylated, apply streptavidin-AP conjugate for 30min
- After last wash in TBST add AP chromogen substrate, 30min at ambient temp.
- Wash slides in distilled water.
- Counterstain with haematoxylin (optional)
- Wash in tap water for at least 15 min and mount with aquamount