## Immunohistochemistry

Tissues fixed in 4% paraformaldehyde and embedded in paraffin (Formaldehyde Fixed and Paraffin Embedded, FFPE)

All steps are done at room temperature, unless otherwise specified

**Preparation of 3 μm slides:**

Dry slides for 30 min in oven at 60℃

Remove paraffin by two 10 min washes in xylene. Remove xylene by 5 min washes subsequently in 100%, 95%, 80% and 75% ethanol.

Rehydrate in PBS for 10 min.

**Antigen retrieval:**

Place slides in citrate buffer pH5 held at 90-98°C for 15 min, then allow to cool to room temp.

Wash slides in PBS, three times 5 min.

**Blocking:**

Block the tissue slides in 3% BSA in PBS for1 hour at 37℃

**Primary antibody labelling:**

Incubate primary antibody overnight at 4℃

Wash slides in PBS, 4 times 10 min

***IMMUNOFLUORESCENCE STAINING***

All steps are carried out in the dark

Incubate fluorophore-conjugated secondary antibody for 1 hour at 37℃

Wash slides in PBS, 3 times 10 min

Incubate slides in DAPI for nuclear counterstain for 10 min

Dehydrate the slides with 5 min washes in subsequently 75%, 80%, 95% and 100% ethanol, then two 5 min washes in 100% xylene.

Cover tissues with either non-aqueous anti-fade mounting media or skip the dehydration steps and use aqueous anti-fade mounting media.

***COLOURIMETRIC STAINING***

Suppress endogenous peroxidase activity with 3% $H_2O_2$ for 12 min

Wash slides in PBS, 3 times 5 min

Incubate HRP-conjugated secondary antibody for 1 hour at 37°C

Wash slides in PBS, 3 times 10 min

Stain the slights with Diaminobenzidine (DAB) for 10-20 min.

Stop the staining with distilled water

Incubate the slides in hematoxylin for nuclear counterstain for 5-10 min

Dehydrate the slides with 5 min washes in subsequently 75%, 80%, 95% and 100% ethanol, then two 5 min washes in 100% xylene.

Cover tissues with neutral balsam mounting media and coverslip