NB100-105 Lot AF-4

Type of samples: pig valve

Deparaffinization

Place the slides in a rack, and perform the following washes:

1. Xylene: 2 x 3 minutes
2. Xylene 1:1 with 100% ethanol: 3 minutes
3. 100% ethanol: 2 x 3 minutes
4. 95% ethanol: 3 minutes
5. 70 % ethanol: 3 minutes
6. 50 % ethanol: 3 minutes
7. Running cold tap water to rinse

Immunohistochemical staining

Day 1

1. Wash the slides 2 x 5 minutes in TBS plus 0.025% Triton X-100 with gentle agitation.
2. Block in 10% normal serum with 1% BSA in TBS for 2 hours at room temperaturature.
3. Drain slides for a few seconds (do not rinse) and wipe around the sections with tissue paper.
5. Apply primary antibody diluted in TBS with 1% BSA – dilution 1/50.
6. Incubate overnight at 4°C.

Day 2

1. Rinse 2 x 5min TBS 0.025% Triton with gentle agitation.
2. We are using an HRP conjugate for detection, so incubate the slides in 0.3% H2O2 in TBS for 15 min (n, blocking of endogenous peroxidise)
3. For enzymatic detection (HRP) we applied enzyme-conjugated secondary antibody to the slide diluted to the concentration recommended by the manufacturer in TBS with 1% BSA, and incubate for 1 hour at room temperature.
4. Rinse 3 x 5min TBS.
5. Develop with chromogen for 10 min at room temperature.
6. Rinse in running tap water for 5 min.
8. Dehydrate, clear and mount.