NBP1-90071 Lot A81346

Type of samples: pig aortic 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

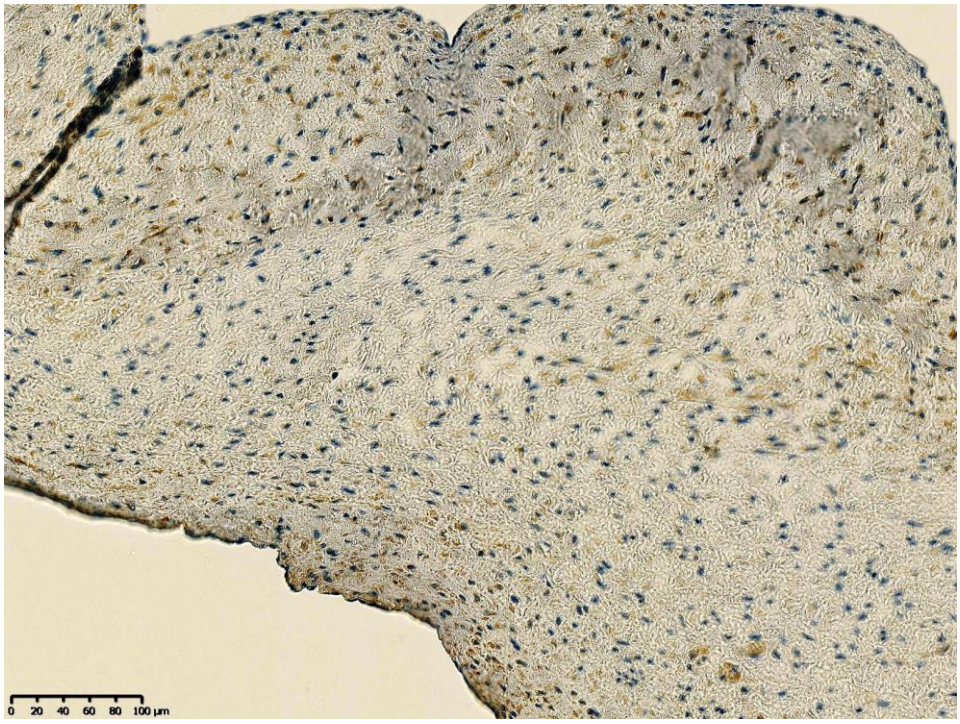
Day 1

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 1/25.
6. Incubate overnight at 4°C.

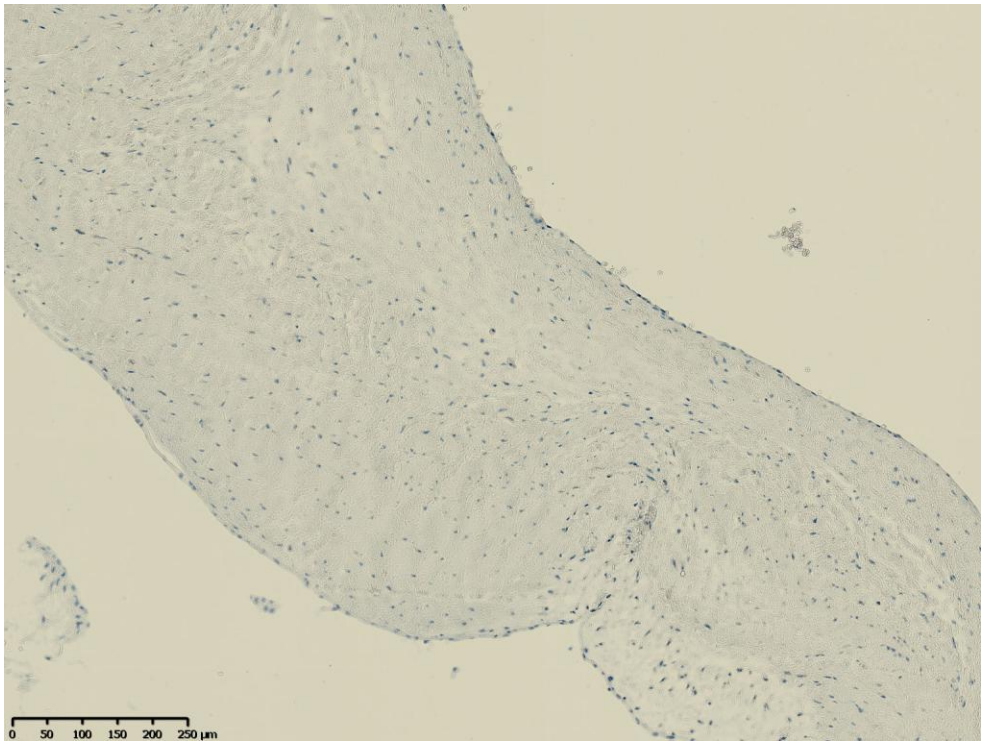
Day 2

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.

CD73 expression is present one the pig aortic valve (picture attached). Staining for negative control for secondary antibodies was performed (picture attached).



Positive CD39 staining in porcine aortic valve cells (brown)



Negative control for secondary antibodies.