

NBP1-85740 Lot A81346

Type of samples: human 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 temperature.
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 1/100.
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 peroxidase)
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.

CD39 expression is present on the human aortic valve (picture attached). Staining for negative control for secondary antibodies was performed (picture attached).



Positive CD73 staining in human aortic valve (brown)



Negative control for secondary antibodies.