

NB300-101 Protocol

Immunohistochemistry Protocol for Neurokinin 1 Receptor Antibody (NB300-101)

[[URL:https://www.novusbio.com/products/nk1r-antibody_nb300-101]][[Caption:NK1R Antibody]]

Immunohistochemistry

- 1) Perfuse rat through the ascending aorta with 500 ml. of 0.1 M phosphate buffered saline (PBS) (pH 7.4 @ 4 degrees C), followed by 750 ml. of PBS containing 4% formaldehyde and 12.5% picric acid (pH 6.9, 4 degrees C). Tissue of interest is then dissected and post-fixed in PBS containing 4% formaldehyde and 12.5% picric acid (pH 6.9 at 4 degrees C) for at least 4 hours, followed by fixation in PBS containing 30% sucrose (pH 7.4, 4 degrees C) for at least 24 hours. Until the tissue sinks.
- 2) Cut fixed sections at 60 um using a sliding microtome.
- 3) If using culture cells, remove media and perform 2-3 washed in PBS (pH 7.4) -use same protocol without agitation.
- 4) Place tissue section in microcentrifuge tubes containing 1 ml. Of PBS, pH 7.4 as they are being cut. Wash sections (in PBS) on an upright rotator for 10-15 minutes.
- 5) Remove PBS and add 1 ml. of blocking solution (PBS + 1% normal donkey serum (NDS) + 0.3% triton X-100).
- 6) Incubate tissue sections in blocking solution for 30 minutes at room temperature (RT) on rotator.
- 7) Remove blocking solution and add anti-neurokinin-1 receptor or anti-Neurokinin-3 receptor(NB 300-101/NB 100-102) antibody +PBS + 1% NDS + 0.3% triton X-100 + NB 300-101/102 @ 1:100~1:1000
- 8) Incubate overnight on rotator at RT.
- 9) Remove primary antisera and perform 3 x 10 minute PBS washes (1ml volume) on rotator.
- 10) Add 1 ml. of secondary antibody and incubate for 2 hours at RT on rotator.
-PBS + 1% NDS + 0.3% triton X 100 + anti-rabbit Cy3 (Jackson Immunoresearch Labs) at 1:600
- 11) Perform 3 X 10 minute PBS washes (1 ml volume) on rotator
- 12) Mount tissue sections on gelatin coated slides and allow tissue to dry
- 13) Run slides through alcohol gradients (70%, 90%, 100%, xylene) leaving slide in each alcohol and xylene for 2 minutes
- 14) Coverslip with DPX mountant (FLUKA) ** Note: 24 well plates may be substituted for microcentrifuge tubes. Use flat top bench rotator instead of upright rotator.