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NB300-102 Protocol

Immunohistochemistry Protocol specific for Neurokinin B Receptor Antibody (NB300-102)

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[[Caption:NK3R/TACR3/Neurokinin B Receptor Antibody]]
Procedure Guide for NB 300-102 Polyclonal Anti-Neurokinin-3 (NK-B) Receptor Immunohistochemistry

1) Tissue of interest is dissected and post-fixed in PBS containing 4% formaldehyde or PBS containing 4% formaldehyde + 0.1% gluteraldehyde**, followed by fixation in PBS containing 20% sucrose, overnight at 4C. Until the tissue sinks.
2) Cut fixed sections at 15 mm or 40 mm using a cryostat or sliding microtome, depending on incubation technique [for cryosections use 15 mm and for free-floating sections use 40 mm].
3) If using culture cells, remove media and perform 2-3 washed in PBS (pH 7.4) -use same protocol without agitation.
4) Wash sections in PBS for 10 minutes, 3 times.
5) Block the tissue with 1% BSA / 1% goat serum / 0.3% triton X-100 in 0.1M PBS, for 1 hour at RT.
6) Dilute anti-NK-3 receptor (NB 300-102) in blocking buffer at 1:1000 for cryosections and 1:5000 for free-floating sections.
7) Incubate sections overnight at 4C.
8) Wash sections in PBS for 10 minutes, 3 times.
9) Dilute secondary antibody (1:400 ?? Alexa 568 [Rho] goat anti-rabbit, from Molecular Probes, Inc.) in blocking buffer and incubate sections for 2 hours at RT.
10) Wash sections in PB (not PBS) for 10 minutes, 3 times.
11) Mount coverslips to slides using Fluoromount-G (Southern Biotech Assoc., cat# 0100-01)

**Note: Mouse dorsal horn and cerebral cortex were used as positive control section.

*Tissues fixed in 4% paraformaldehyde + 0.1% gluteraldehyde are used for EM analysis