ADVANCED TARGETING SYSTEMS

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Antibody to NK-1 Receptor
RABBIT POLYCLONAL

Catalog Number: AB-N04

Immunohistochemistry Protocol:

1. Perfuse rat through the ascending aorta with 500 ml of 0.1 M phosphate-buffered saline (PBS) (pH 7.4, 4°C), followed by 750 ml of PBS containing 4% formaldehyde and 12.5% picric acid (pH 6.9, 4°C). Tissue of interest is then dissected and post-fixed in PBS containing 4% formaldehyde and 12.5% picric acid (pH 6.9, 4°C, at least 4 hours), followed by fixation in PBS containing 30% sucrose (pH 7.4, 4°C, at least 24 hours).
2. Cut fixed sections at 60 mm using a sliding microtome.
3. If using cultured cells, remove media and perform 2-3 washes in phosphate buffered saline (PBS, pH 7.4) -- Use same protocol without agitation.
4. Place tissue sections in microcentrifuge tubes containing 1 ml of phosphate-buffered saline (PBS, pH 7.4) as they are being cut. Wash sections (in PBS) on an upright rotator (Glas-Col) for 10-15 min.
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 min at room temp on rotator.
7. Remove blocking solution and add NK-1r antiserum. PBS + 1 % NDS + 0.3% triton + NK-1r antiserum @ 1:5000
8. Incubate overnight on rotator at room temp.
9. Remove primary antisera and perform three 10-min PBS washes (1 ml volume) on rotator.
10. Add 1 ml of secondary antibody and incubate for 2 hours at room temp on rotator. PBS + 1% NDS + 0.3% triton + anti-rabbit Cy3 (Jackson Immunoresearch labs) @ 1:600
11. Perform three 10-min 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 can be substituted for the microcentrifuge tubes.
Use flat top bench rotator instead of upright rotator.