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SHEEP ANTI-NERVE GROWTH FACTOR - BETA AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NO: AB1528P

LOT NUMBER:

QUANTITY: 50 µg

SPECIFICITY: Nerve growth factor-beta (NGF). Less than 1% cross-reactivity with recombinant human BDNF and NT4 and approximately 2% with NT3 by ELISA.

IMMUNOGEN: Mouse nerve growth factor-beta purified from salivary glands.

APPLICATIONS: Immunohistochemistry: 1-10 µg/mL (see suggested protocol).
Immunoblotting: 1-10 µg/mL
Inhibition of biological activity *in vitro*: 1-10 µg/mL
ELISA: 1-10 µg/mL
In vivo animal studies: use neat.
Optimal working dilutions must be determined by the end user.

BIOLOGICAL ACTIVITY: This antibody will completely inhibit the survival and neurite outgrowth actions of mouse NGF in chicken dorsal root ganglion neurons *in vitro*.

SPECIES REACTIVITY: Reacts with mouse, rat, chicken and human NGF-beta. Does not react with bovine NGF. Other species have not been tested.

FORMAT: Affinity purified sheep immunoglobulin

PRESENTATION: Lyophilized from 10 mM PBS. Reconstitute with 50 µL of sterile distilled water.

STORAGE/HANDLING: Maintain lyophilized material at -20 to -70°C for up to one year after date of receipt. After reconstitution maintain at -20 to -70°C in undiluted aliquots for up to six months. Avoid repeated freeze-thaw cycles.

REFERENCES: Zhou et al., *J. Neurosci. Meth*. (1994) **54**:95.
Liu et al, *J. Neurochem.* (1996) **66**:2995.

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NEUROTROPHIN IMMUNOHISTOCHEMISTRY

The neurotrophins have proved difficult to localize which may be due to masking by, for example, their association with the *trk* receptors or very low concentrations. Where success has been achieved, the conditions required vary greatly for different tissues. A protocol has been included for central nervous system tissue and, while it is similar to commonly used methods, it is important to give strict attention to details such as thorough washing, fixative and detergent concentrations, concentration and quality of the primary antibodies and length of incubations, etc. You may find it possible to use alternate protocols, however we have experienced many failures using variations of the current protocol (and some failures when strict adherence to the procedure is maintained). A suitable procedure to stain nerve terminals is still being developed. Neurotrophin receptors have proved much easier to localize.

It is recommended that you include a few sections of adult rat cerebellum, spinal cord or kidney in each experiment since these are tissues which are the easiest to stain.

Protocol for Immunohistochemistry in the Central Nervous System

At all steps thorough washing is necessary to reduce background.

Fixation

Animals are perfused with 1% sodium nitrite in phosphate buffered saline (PBS) (about 50 mL) followed by 1 liter of Zamboni's fixative (4% formaldehyde, 15% picric acid in 0.1M phosphate buffer). Post fix for no longer than 2 hours.

Tissue Preparation

Tissues are removed and washed briefly with PBS followed by cryoprotection in 30% sucrose in PBS overnight at 4°C. 30 mM cryostat sections are cut and washed with agitation in:

PBS	(1x15min)
50% ethanol	(3x15min)
PBS	(1x15min)

Sections can be stored at 4°C in TBS for several weeks in the presence of 0.2% sodium azide.

Blocking and Primary Antibody Incubations

A 24 well tissue culture plate works well for incubations. Sections are blocked with 20% normal horse serum in PBS for at least 1 hour followed by incubation with primary antibody diluted in 2xPBS, 0.3% Triton X-100 containing 0.02% sodium azide. Incubation can range from 24 hrs to 1 week. Room temperature (RT) is used for 24 hr incubations but 4°C is used for longer incubations.
Prepare sufficient antibody only to cover the sections.

Secondary Antibodies

Primary antibodies are removed and sections are washed in PBS-T (PBS + 0.1% Tween), 3x15min. Biotinylated affinity purified IgG antibody is then applied to the sections for 2 hours at RT followed by another 3x15 min washes in PBS-T.

ABC Reagent: The ABC reagent is prepared 30 minutes prior to use and applied to sections for 2 hours at RT according to the manufactures recommendation.

ABC reagent is then removed and sections are washed with Tris buffered saline (TBS), 3x15 mins, to remove all traces of the ABC.

Development

0.25% nickel sulfate/TBS solution is prepared and to 20 mls of this solution, 10 mg Diaminobezidine (DAB) is added. Immediately prior to use, 7.5 mL of hydrogen peroxide (30% w/v) is added to this solution. Sections are incubated in this solution for up to 30 minutes, until the bluish color develops. If sections show rapid color change due to high background, the neurotrophin immunoreactivity will be difficult to detect. Washing procedure will then need to be improved.

To end the reaction, remove the DAB solution and wash sections in TBS, 3x15 mins. Transfer sections to glass microscope slides, stretch and arrange using a small paintbrush. Slides are then air dried, dehydrated through graded alcohols, cleared in xylene, and mounted in a xylene based mounting media.

Sections can then be examined using light microscopy.

Protocol for Neurons in the Peripheral Nervous System

See Zhou et al., (1994) *J. Neuroscience Methods* **54**:95-102. This protocol works well for neurons in cranial and spinal ganglia as well as for those in sympathetic ganglia.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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