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Product datasheet for PP1000P2

BDNF Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, FN, IF, IHC, WB
Recommended Dilution:	**Sandwich ELISA:** To detect Human BDNF by Sandwich ELISA (using 100 µl/well antibody solution) a concentration of 0.5-2.0 µg/ml of this antibody is required. This antibody, in conjunction with a biotinylated anti-BDNF (*Cat.-No* PP1000B1 or PP1000B2) as a detection antibody, allows the detection of at least 2000-4000 pg/well of recombinant Human/Mouse/Rat BDNF. **Western blot:** To detect Human BDNF by Western blot analysis this antibody can be used at a concentration of 0.1-0.2 µg/ml. The detection limit for recombinant Human/Mouse/Rat BDNF is 1.5-3.0 ng/lane, under either reducing or non-reducing conditions. **Immunohistochemistry** (See Protocols). This antibody stained U-2 OS and U-251 MG cells. The primary antibody was incubated at 2.0 µg/ml overnight at 4°C followed by a fluorescent labeled secondary antibody. I*nformation and photo are courtesy of the Cell Profiling group, SciLifeLab Stockholm.* **Neutralization:** To yield one-half maximal inhibition ND_{50} of the biological activity of Human/Mouse/Rat BDNF (2.0 µg/ml) a concentration of 0.54-0.81 µg/ml of this antibody is required.
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	Highly purified (>98%) E.coli derived, 27.0 kDa recombinant Human/Mouse/Rat BDNF (*Cat.-No* PA047)
Specificity:	The antibody recognizes BDNF.
Formulation:	PBS, pH 7.2, without preservatives State: Aff - Purified State: Lyophilized purified (sterile filtered) Ig fraction
Reconstitution Method:	Restore in sterile water to a concentration of 0.1-1.0 mg/ml.
Purification:	Affinity Chromatography employing an immobilized Human/Mouse/Rat BDNF matrix
Conjugation:	Unconjugated

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Storage: Prior to reconstitution store at 2-8°C or frozen at -20° to -80°C.
Following reconstitution the antibody can be stored undiluted at 2-8°C for two weeks or (in aliquots) at -20°C for up to six months.
Avoid repeated freezing and thawing.

Stability: Shelf life: Five years from despatch

Database Link: Entrez Gene 627 Human
P23560

Background: Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors that includes NGF, NT3, and NT4. All neurotrophins have six conserved cysteine residues and share a 55% sequence identity at the amino acid level. BDNF is a potent neurotrophic factor that supports the growth and survivability of nerve and/or glial cells. BDNF has been shown to enhance the survival and differentiation of several classes of neurons in vitro, including neural crest and placode derived sensory neurons, dopaminergic neurons in the substantia nigra, basal forebrain cholinergic neurons, hippocampal neurons, and retinal ganglial cells. BDNF is expressed within peripheral ganglia and is not restricted to neuronal target fields, raising the possibility that BDNF has paracrine or even autocrine actions on neurons as well as non neuronal cells. Expression of BDNF is reduced in both Alzheimer's and Huntington disease patients. In addition, functional studies showed that age-associated changes in BDNF-mediated pathways can enhance inflammation and increase myocardial injury after myocardial infarction in the aging heart.

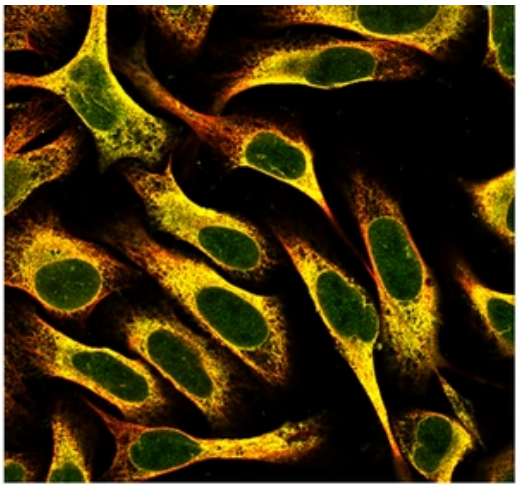
Synonyms: Brain-derived neurotrophic factor, Abrineurin

Note: Protocol: **Neutrophin 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 µm 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.
Neurotrophin antibodies are normally used after affinity purification at a concentration of approximately 0.5-1.0 µg/mL. 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 sulphate/TBS solution is prepared and to 20 mls of this solution, 10 mg Diaminobezidine (DAB) is added. Immediately prior to use, 7.5 (l 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 prodcedure 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.

Product images:



Staining U-2 OS and U-251 MG cells using BDNF Antibody Cat.-No [PP1000P]