ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Human Brain derived neurotrophic factor (BDNF) Antibody

☐ Cat# BDNF12-A	Rabbit anti-Human BDNF pAb, affinity purified	**SIZE**: 100 ug
☐ Cat# BDNF12-P	Control/Blocking peptide for BDNF antibody	**SIZE**: 250 ug

During development, promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous systems. Participates in axonal growth, pathfinding and in the modulation of dendritic growth and morphology. Major regulator of synaptic transmission and plasticity at adult synapses in many regions of the CNS. The versatility of BDNF is emphasized by its contribution to a range of adaptive neuronal responses including long-term potentiation (LTP), long-term depression (LTD), certain forms of short-term synaptic plasticity, as well as homeostatic regulation of intrinsic neuronal excitability.

Source of Antigen or Antibodies

Uniprot: P23560

Host: Rabbit

Form: Polyclonal, affinity purified over a peptide agarose column

Immunogen: Mix of 2 peptides between 13-14 amino acids in length from the mature region of Human BDNF

Species Reactivity: Human

Cross reactivity: 100% homology between Mouse, Rat, Monkey, Bovine, Sheep, Goat, Chicken, Pig, Cat, Dog, Camel, Zebrafish, and more species not listed.

Subcellular Location: Extracellular region or secreted

Alternative names: Brain-derived neurotrophic factor, BDNF, Abrineurin

Tissue specificity: Brain. Highly expressed in hippocampus, amygdala, cerebral cortex, and cerebellum. Also expressed in heart, lung, skeletal muscle, testis, prostate, and placenta.

Isoforms: Isoform 2- 29kDa. Isoform 3-30kDa. Isoform 4-37 kDa. Isoform 5- 31 kDa.

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP

Negative Control: Non immune Rabbit IgG (**ADI cat# 20009-1**). Blocking peptide (**ADI cat# BDNF12-P),** use 5-10 ug of control peptide per 1 ug of affinity pure antibody or 1 ul antiserum to confirm specificity.

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG
☐ _____ ul solution; Concentration: _____
Supplied in PBS pH 7.4 +0.05% Azide

☐ lyophilized powder
Reconstitute powder in 200 ul PBS to 0.5 mg/ml

Control/blocking peptide
☐ 250 ug/250 ul ☐ solution ☐ lyophilized powder
Reconstitute powder in PBS at 1 mg/ml.

Storage:

Short-term: 4^{o}C for 1 month

Long-term: at –20^{0}C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple free/thaw cycles or store working, diluted solutions.

Recommended Usage

ELISA: Assay dependent concentration. Typically 0.1-2.0 ug/ml for capture/detection antibodies.

Western Blotting: 1-3 ug/ml using affinity pure.
Theoretical band size: 14 kDa for the mature protein and ~28-36 kDa for pro-BDNF has been reported.
Observed band size: ~15-17 kDa.

IHC: 2-5 ug/ml. Antigen retrieval does not need to be performed.

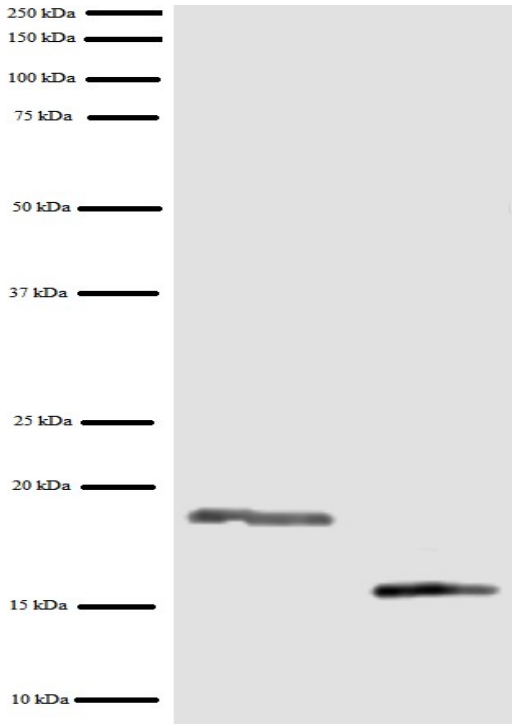
*Above concentrations are a suggestion and user's must optimize assay based on their conditions. Antibody may work in other applications such as Flow Cytometry or IP. These methods have not been tested by ADI.

**This product is for In vitro research use only.*

Related materials available from ADI

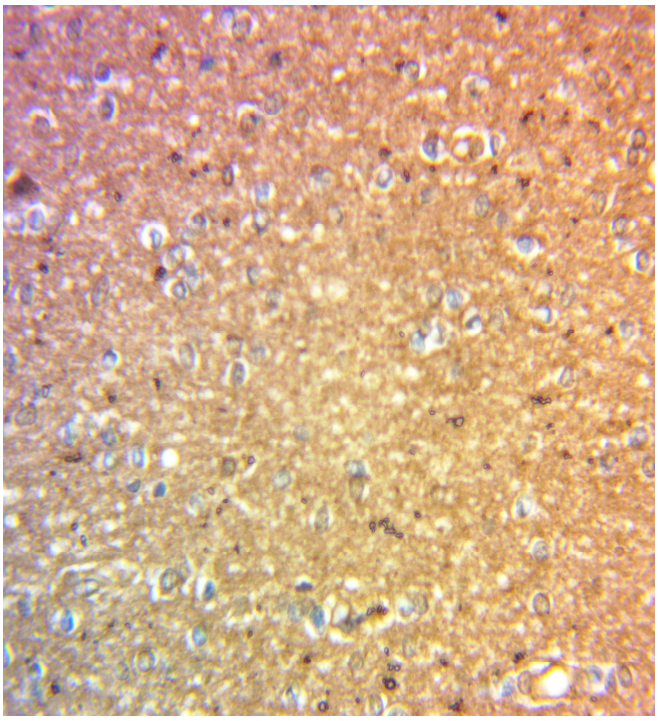
Catalog#	Description
BDNF11-R	Recombinant (E.Coli) Human Brain Derived Neurotrophic Factor (BDNF) Protein
BDNF11-C	Purified Human Brain Derived Neurotrophic Factor (BDNF) Protein Control for Western
BDNF11-M	Monoclonal Anti-Human Brain Derived Neurotrophic Factor (BDNF) IgG #1, aff. Pure

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25 ug of total Human brain lysate (Lane#1) and total Mouse brain lysate (Lane #2) were heated for 5 minutes at 95ºC then loaded onto an "Any kD" SDS-PAGE gel (Biorad). Gels were run for ~1 hour and 30 minutes at 100V and transferred to a 0.2 um nitrocellulose membrane using the low MW settings on a Transblot Turbo (Biorad). Ladders were annotated with ADI antigen-antibody pen and blocked for 1 hour at room temperature with 1% Fish plasma (Aquablolock, EastCoastBio). BDNF12-A was diluted with TBST+3% BSA to 1 ug/ml and incubated overnight at 4ºC. Blot was washed with TBS-T 3 times for 5 minutes. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was added at 1:5000 dilution (0.1 ug/ml) for 1 hour at room temperature. Blot was washed 3 times with TBS-T for 5 minutes. ADI Femto ECL substrate (**ADI cat#80210**) was added for 5 minutes and the blot was imaged on a LI-COR C-digit high sensitivity settings. Specific band is observed at ~17 kDa in Human brain and ~15 kDa in mouse brain

Western Blot Positive control: Brain lysate



Human brain was fixed in 10% phosphate buffered formalin overnight, cut to 8 um, and mounted on Superfrost ®Plus slides. Slides were baked for 15 minutes at 60ºC then deparaffinized and washed in DI water then PBST for 5 minutes each. Tissue was blocked in 2.5% normal goat serum for 30 minutes at room temperature. BDNF12-A was diluted to 5 ug/ml in PBST+1% BSA and incubated overnight at 4ºC. The slide was then washed 3 times for 5 minutes each in PBST. 3% Hydrogen peroxide was added for 10 minutes then rinsed twice in PBST. Goat anti-rabbit IgG-HRP polymer (Vectorlabs, cat#MP-7451) was added for 30 minutes at room temperature then washed 3 times in PBST. DAB was added for 1 minute then washed with distilled water and counterstained with Gill II Hematoxylin for 3 minutes. The slide was rinsed in running tap water then dipped in 0.1% sodium bicarbonate pH 9.6 for 30 seconds then washed in distilled water. Slide was mounted with Advantage aqueous mounting medium and coverslipped.