**Product datasheet**

**Anti-BDNF antibody [EPR1292] ab108319**

- **Product name**: Anti-BDNF antibody [EPR1292]
- **Description**: Rabbit monoclonal [EPR1292] to BDNF
- **Host species**: Rabbit
- **Specificity**: This product may cross react with the following family members: NGF beta, neurotrophin 3, neurotrophin 4. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.

**Tested applications**

- Suitable for: Flow Cyt (Intra), WB, IHC-P, IHC-Fr, ICC/IF

**Species reactivity**

- Reacts with: Mouse, Rat, Human

**Immunogen**

- Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab182199)

**Positive control**

- WB: Human, rat and mouse brain, hippocampus and cerebellum lysates; IHC-P: Human brain tissue, human bladder cancer tissue; ICC/IF: HeLa cells; Flow Cyt (intra): HeLa cells; IHC-Fr: Mouse and Rat cerebrum tissue, Hu cerebral cortex.

**General notes**

For BDNF, multiple WB bands are possible and expected. The human protein has 5 isoforms (precursors: 28 – 37 kDa) and can be glycosylated (Uniprot: http://www.uniprot.org/uniprot/P23560). The mature form is expected at ~14 kDa (monomer) and the dimer at ~28 kDa.

This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**Properties**

- **Form**: Liquid
- **Storage instructions**: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

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Function
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.

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

Involvement in disease
Bulimia nervosa 2
Congenital central hypoventilation syndrome
Sequence similarities
Belongs to the NGF-beta family.

Post-translational modifications
The propeptide is N-glycosylated and glycosulfated.
Converted into mature BDNF by plasmin (PLG).

Cellular localization
Secreted.

Images

All lanes: Anti-BDNF antibody [EPR1292] (ab108319) at 1/1000 dilution (unpurified)

Lane 1: Human hippocampus lysate
Lane 2: Rat hippocampus lysate
Lane 3: Mouse hippocampus lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Gt anti Rb IR680 at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa
Additional bands at: 15 kDa (possible mature (processed) protein), 28 kDa (possible multimer), 35 kDa, 45 kDa (possible immature (unprocessed)). We are unsure as to the identity of these extra bands.

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with unpurified ab108319 (1/1000) overnight at 4°C. Ab8245 (mouse anti-GAPDH; 0.05 ug/mL) was included as a loading control. Antibody binding was detected using goat anti-rabbit IgG IR-680 (green) and goat anti-mouse IgG IR800 (red) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx
**Western blot - Anti-BDNF antibody [EPR1292]**

*ab108319*

**All lanes**: Anti-BDNF antibody [EPR1292] (ab108319) at 1/1000 dilution (Purified)

**Lane 1**: Human brain lysates with 5% NFDM/TBST
**Lane 2**: Mouse brain lysates with 5% NFDM/TBST
**Lane 3**: Rat brain lysates with 5% NFDM/TBST
**Lane 4**: Human hippocampus lysates with 5% NFDM/TBST
**Lane 5**: Mouse hippocampus lysates with 5% NFDM/TBST
**Lane 6**: Rat hippocampus lysates with 5% NFDM/TBST
**Lane 7**: Human cerebellum lysates with 5% NFDM/TBST
**Lane 8**: Mouse cerebellum lysates with 5% NFDM/TBST
**Lane 9**: Rat cerebellum lysates with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

**Predicted band size**: 15 kDa

**Observed band size**: 15-45 kDa

IHC image of BDNF staining in a section of frozen normal human cerebral cortex performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108319, 1/200 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
Immunohistochemistry (Frozen sections) analysis of rat cerebral cortex tissue sections labeling BDNF with Purified ab108319 at 1/100 (2.8 µg/ml). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder cancer tissue sections labeling BDNF with Purified ab108319 at 1:500 dilution (0.56 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0)
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling BDNF with purified ab108319 at 1/30 dilution (10µg/ml) (red). Cells were fixed with 80% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Different batches of ab108319 were tested on Mouse brain lysate at 0.3 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 14-45 kDa.

Immunohistochemistry (Frozen sections) analysis of mouse cerebrum tissue sections labeling BDNF with Purified ab108319 at 1/100 (2.8 µg/ml). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.
Immunocytochemistry/Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling BDNF with Purified ab108319 at 1:500 (0.6 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunohistochemical analysis of paraffin-embedded human brain tissue using unpurified ab108319 at 1/100 dilution.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling BDNF with unpurified ab108319 at a dilution of 1/750. Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton-X100 in PBS. ab150081 (1/200) was used as the secondary antibody.
The antibody produces a strong, golgi-associated labelling pattern in both PF and MeOH fixed samples.
Anti-BDNF antibody [EPR1292] (ab108319)

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