Anti-BDNF Antibody [JE59-35]

HA722912

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IHC-Fr, FC
Molecular Wt:	Predicted band size: 28 kDa
Clone number:	JE59-35

Description: BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support survival of existing neurons, and encouraging growth and differentiation of new neurons and synapses. In the brain it is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory, and higher thinking. BDNF is also expressed in the retina, kidneys, prostate, motor neurons, and skeletal muscle, and is also found in saliva. BDNF itself is important for long-term memory. Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells in a process known as neurogenesis. Neurotrophins are proteins that help to stimulate and control neurogenesis, BDNF being one of the most active. Mice born without the ability to make BDNF have developmental defects in the brain and sensory nervous system, and usually die soon after birth, suggesting that BDNF plays an important role in normal neural development. Other important neurotrophins structurally related to BDNF include NT-3, NT-4, and NGF. BDNF is made in the endoplasmic reticulum and secreted from dense-core vesicles. It binds carboxypeptidase E (CPE), and disruption of this binding has been proposed to cause the loss of sorting BDNF into dense-core vesicles. The phenotype for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar abnormalities and an increase in the number of sympathetic neurons. Certain types of physical exercise have been shown to markedly (threefold) increase BDNF synthesis in the human brain, a phenomenon which is partly responsible for exercise-induced neurogenesis and improvements in cognitive function. Niacin appears to upregulate BDNF and tropomyosin receptor kinase B (TrkB) expression as well.

Immunogen:	Recombinant protein.
Positive control:	U-87 MG cell lysate, HeLa cell lysate, C6 cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, U-87 MG, PC-12, human brain tissue, mouse brain tissue, mouse hippocampus tissue, rat brain tissue, rat hippocampus tissue.
Subcellular location:	Secreted.
Database links:	SwissProt: P23560 Human | P21237 Mouse | P23363 Rat
Recommended Dilutions:	
WB	1:2,000
IF-Cell	1:500
IHC-P	1:2,000
IHC-Fr	1:500
FC	1:1,000
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

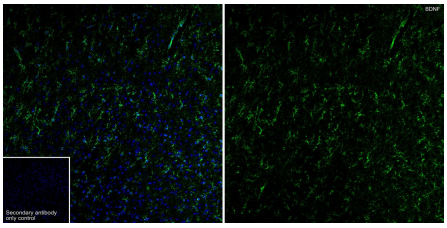

Fig1: Immunofluorescence analysis of frozen mouse cerebral cortex tissue with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722912, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

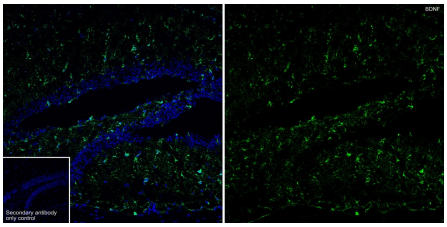

Fig2: Immunofluorescence analysis of frozen mouse hippocampus tissue with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722912, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

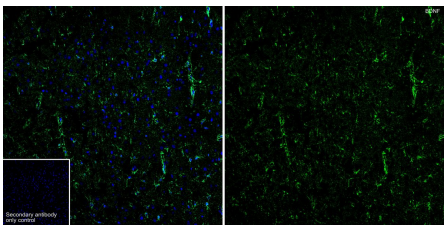

Fig3: Immunofluorescence analysis of frozen rat cerebral cortex tissue with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722912, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

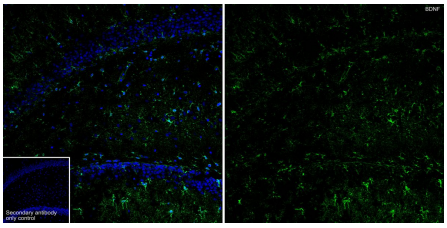

Fig4: Immunofluorescence analysis of frozen rat hippocampus tissue with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722912, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

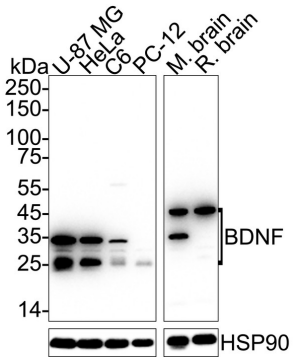

Fig5: Western blot analysis of BDNF on different lysates with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

Lane 1: U-87 MG cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: C6 cell lysate (20 µg/Lane)
Lane 4: PC-12 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (40 µg/Lane)
Lane 6: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 28 kDa
Observed band size: 25/35/45 kDa

Exposure time: 25 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722912) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

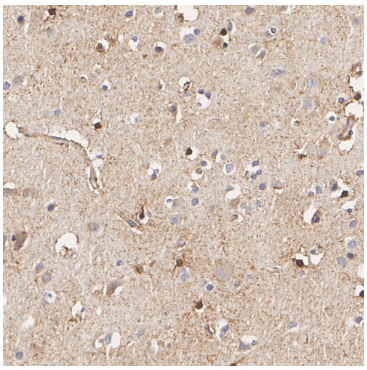

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722912) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

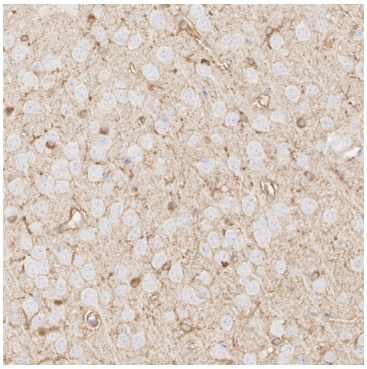

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722912) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

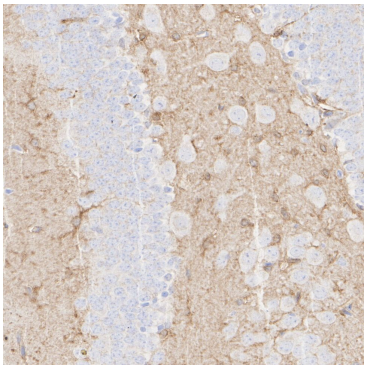

Fig8: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722912) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

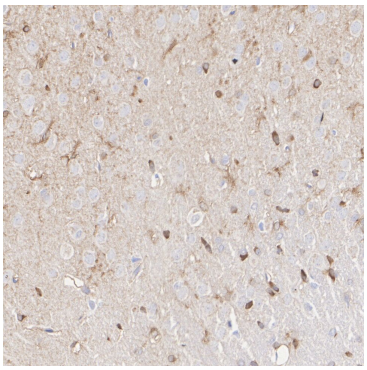

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722912) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

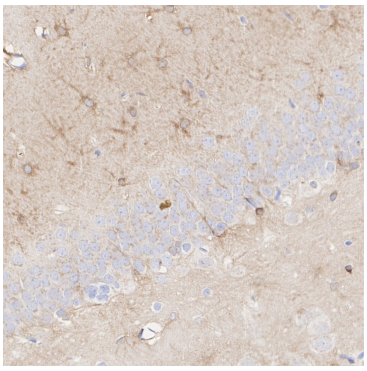

Fig10: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-BDNF antibody (HA722912) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722912) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

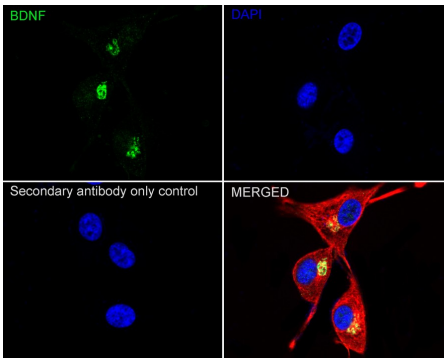

Fig11: Immunocytochemistry analysis of U-87 MG cells labeling BDNF with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

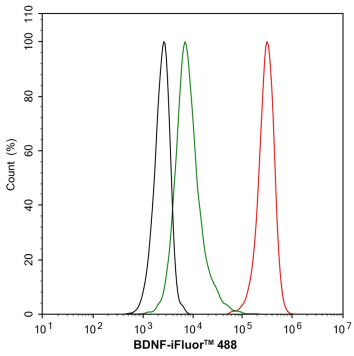

Fig12: Flow cytometric analysis of U-87 MG cells labeling BDNF.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722912, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

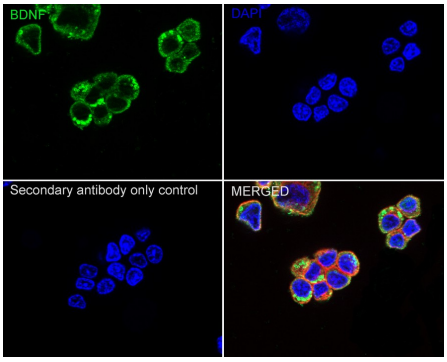

Fig13: Immunocytochemistry analysis of PC-12 cells labeling BDNF with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-BDNF antibody (HA722912) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

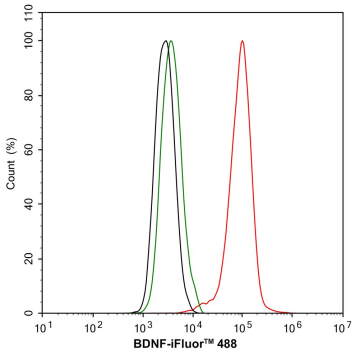

Fig14: Flow cytometric analysis of PC-12 cells labeling BDNF.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722912, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Palasz E et al. BDNF as a Promising Therapeutic Agent in Parkinson's Disease. Int J Mol Sci. 2020 Feb
2. Colucci-D'Amato L et al. Neurotrophic Factor BDNF, Physiological Functions and Therapeutic Potential in Depression, Neurodegeneration and Brain Cancer. Int J Mol Sci. 2020 Oct