

Product Datasheet

BDNF Antibody (14C8.1D9)

NBP2-42215

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

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NBP2-42215

BDNF Antibody (14C8.1D9)

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	14C8.1D9
Preservative	0.02% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	627
Gene Symbol	BDNF
Species	Human, Rat
Reactivity Notes	Immunogen has 100% homology with mouse, rat, bovine, porcine, and monkey. Rat data from customer review.
Immunogen	Partial recombinant human BDNF protein (between amino acids 100-247) [UniProt P23560]

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 50 ug/ml, Immunohistochemistry 10-15 ug/ml, Immunocytochemistry/Immunofluorescence 20 ug/ml, Immunohistochemistry-Paraffin 10-15 ug/ml
Application Notes	BDNF is a 247 amino acids long protein with predicted molecular weight of 27.8 kDa, however, it undergoes molecular processing / cleavage as well as glycosylation and disulphide bond formation. Cleaved BDNF is ~14 kDa while the glycosylated form as well as the dimers may run at relatively higher molecular weight position in WB assay.



Publications

Wei X, Ma W, Gu H et al. Intra-amniotic mesenchymal stem cell therapy improves the amniotic fluid microenvironment in rat spina bifida aperta fetuses Cell proliferation 2022-10-20 [PMID: 36266504] (IHC-Fr, Rat)

Wu KJ, Chen YH, Bae EK et al. Human Milk Oligosaccharide 2'-Fucosyllactose Reduces Neurodegeneration in Stroke Brain Transl Stroke Res 2020-01-02 [PMID: 31898186]

Luo S, Su Kang S, Wang ZH et al. Akt Phosphorylates NQO1 and Triggers its Degradation, Abolishing its Antioxidative Activities in Parkinson's Disease J. Neurosci. 2019-07-29 [PMID: 31358653]

Wang ZH, Xiang J, Liu X et al. Deficiency in BDNF/TrkB Neurotrophic Activity Stimulates delta-Secretase by Upregulating C/EBP beta in Alzheimer's Disease Cell Rep 2019-07-16 [PMID: 31315045] (WB, KD, Mouse, Human, Rat)

Xiang, J;Wang, ZH;Ahn, EH;Liu, X;Yu, SP;Manfredsson, FP;Sandoval, IM;Ju, G;Wu, S;Ye, K; Delta-secretase-cleaved Tau antagonizes TrkB neurotrophic signalings, mediating Alzheimer's disease pathologies Proc. Natl. Acad. Sci. U.S.A. 2019-04-19 [PMID: 31004063] (ICC/IF, Mouse)

Wang ZH, Wu W, Kang SS et al. BDNF inhibits neurodegenerative disease-associated asparaginyl endopeptidase activity via phosphorylation by AKT JCI Insight 2018-08-23 [PMID: 30135302] (ICC/IF, Rat)

Lambert WS, Carlson BJ, Formichella CR et al. Oral Delivery of a Synthetic Sterol Reduces Axonopathy and Inflammation in a Rodent Model of Glaucoma. Front Neurosci. 2017-02-22 [PMID: 28223915] (IF/IHC, Rat)



Procedures

Western Blot Protocol for BDNF Antibody (NBP2-42215)

Reagents needed:

- a. Washing Buffer: Tris Buffer Saline with 0.01% of tween 20).
- b. Blocking Buffer: 5% skimmed milk powder in washing buffer).
- c. Secondary antibody, Horseradish peroxidase conjugated.
- d. Chemiluminescent solution (SuperSignal WestPico™, Pierce).

Western blot Method:

1. Perform SDS-PAGE using PVDF membrane. Cut into strips.
2. Activate strips with methanol by dipping them into methanol for 5 min.
3. Discard the methanol and take fresh methanol to repeat step b.
4. Let the strips dry, and then add blocking solution and incubate at RT in a shaker for 30-45 minutes.
5. Dilute primary antibody in blocking buffer. Incubate the number of strips required with the diluted primary antibody at room temperature for 2 hours in a shaker.
6. Wash strips two times with washing buffer at 30 minutes intervals.
7. Dilute HRP conjugated secondary antibody in blocking buffer. Add diluted secondary antibody to the membrane strips and incubate for exactly 1 hour while shaking at RT.
8. Wash the strips with washing buffer for 2-3 hours with 3 to 4 changes on a shaker. This helps in reducing the back ground staining.
9. Prepare the chemiluminescent solution (SuperSignal WestPico™) by mixing solution A and Solution B at 1:1. Mix well. Soak the strip in the chemiluminescent solution; keep for 3-5 minutes under constant shaking.
10. Expose the membrane to a sheet of film and develop.

Immunocytochemistry/Immunofluorescence Protocol for BDNF Antibody (NBP2-42215)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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