

Product Datasheet

Synaptophysin Antibody - BSA Free NBP2-25170

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NBP2-25170

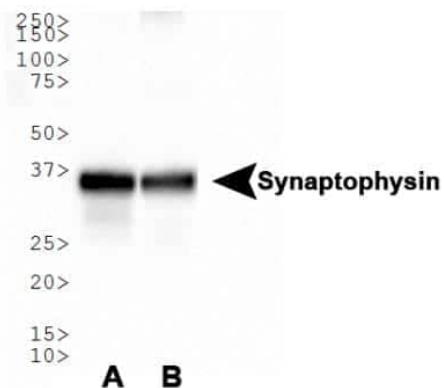
Synaptophysin Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Product Description	
Host	Rabbit
Gene ID	6855
Gene Symbol	SYP
Species	Human, Mouse, Primate
Marker	Pre-synaptic marker
Immunogen	A synthetic peptide made to an C-terminal portion of the human Synaptophysin protein (between residues 230-313) [UniProt# P08247]
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0 ug/ml, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:250, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen reported in scientific literature (PMID 31117227)

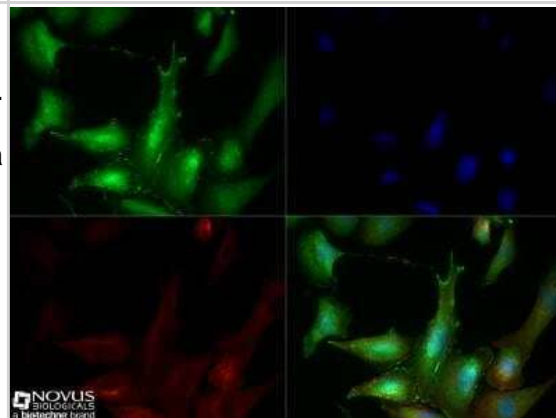


Images

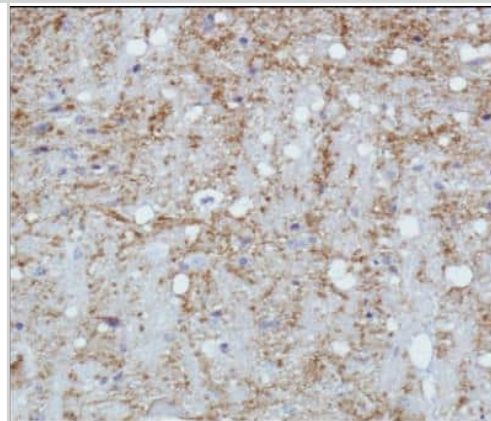
Western Blot: Synaptophysin Antibody [NBP2-25170] - Analysis of Synaptophysin in (A) human brain lysate and (B) mouse brain lysate.



Immunocytochemistry/Immunofluorescence: Synaptophysin Antibody [NBP2-25170] - PC12 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Synaptophysin at a 1:50 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry: Synaptophysin Antibody [NBP2-25170] - Analysis of Synaptophysin in mouse brain.



Publications

Lima BHM, Bombeiro AL, Cartarozzi LP, Oliveira ALR. The Time Course of MHC-I Expression in C57BL/6J and A/J Mice Correlates with the Degree of Retrograde Gliosis in the Spinal Cord following Sciatic Nerve Crush Cells 2022-11-22 [PMID: 36496969]

Toro CA, Hansen J, Siddiq MM et al. The Human ApoE4 Variant Reduces Functional Recovery and Neuronal Sprouting After Incomplete Spinal Cord Injury in Male Mice *Frontiers in Cellular Neuroscience* 2021-02-18 [PMID: 33679326]

He B, Gao R, Lv S et al. Cancer cell employs a microenvironmental neural signal trans-activating nucleus-mitochondria coordination to acquire stemness *Signal transduction and targeted therapy* 2023-07-19 [PMID: 37463926] (ICC/IF, Human)

Huynh B Brainstem Neurotransmitter System Deterioration in Parkinson's Disease and Parkinson's Disease with Dementia Thesis 2023-01-01 (WB, Human)

Ignarro RS, Bombeiro AL, Chiarotto GB et al. Interferon-beta induces major histocompatibility complex of class I (MHC-I) expression and a proinflammatory phenotype in cultivated human astrocytes *Differentiation; research in biological diversity* 2022-10-15 [PMID: 36306693] (FLOW, Rat)

Details:

Dilution used in Flow 1:100

Ramasubramanian B, Griffith C, Hanson M et al. Protective Effects of Chaya against Mitochondrial and Synaptic Toxicities in the Type 2 Diabetes Mouse Model TallyHO Cells 2022-02-21 [PMID: 35203393] (WB, IF/IHC, Mouse)

Cartarozzi LP, Perez M, Fernandes GG et al. Neuroprotection and gliosis attenuation by intravenous application of human mesenchymal stem cells (hMSC) following ventral root crush in mice *Molecular and cellular neurosciences* 2021-12-24 [PMID: 34954382] (IF/IHC)

Awasthi S, Hindle A, Sawant NA Et al. RALBP1 in Oxidative Stress and Mitochondrial Dysfunction in Alzheimer's Disease *Cells* 2021-11-10 [PMID: 34831336]

Chavez-Valdez R, Lechner C, Emerson P et al. Accumulation of PSA-NCAM marks nascent neurodegeneration in the dorsal hippocampus after neonatal hypoxic-ischemic brain injury in mice *J. Cereb. Blood Flow Metab.* 2020-07-23 [PMID: 32703109]

Bernardes D, Rodrigues de Oliveira AL Regular Exercise Modifies Histopathological Outcomes of Pharmacological Treatment in Experimental Autoimmune Encephalomyelitis *Frontiers* 2019-11-20 [PMID: 30524355] (IHC-P, Mouse)

Cartarozzi LP, Perez M, Kirchhoff F, Oliveira ALR Role of MHC-I Expression on Spinal Motoneuron Survival and Glial Reactions Following Ventral Root Crush in Mice *Cells* 2019-05-21 [PMID: 31117227] (IHC-Fr, Mouse)

Procedures

Western Blot protocol for Synaptophysin Antibody (NBP2-25170)

Synaptophysin Antibody:

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-Synaptophysin primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/ Immunofluorescence Protocol for Synaptophysin Antibody (NBP2-25170)

Synaptophysin Antibody:

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.

Immunohistochemistry protocol for Synaptophysin Antibody (NBP2-25170)

Synaptophysin Antibody:

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.



Flow (Intracellular) Protocol for Synaptophysin Antibody (NBP2-25170)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

Optional: Perform cell surface staining as described in the previous section.

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 μ L/ 1×10^6 cells of primary antibody or 1-3 μ L/ 1×10^6 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 μ L per sample and proceed with flow analysis.





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Products Related to NBP2-25170

NB820-59177	Human Brain Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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