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Endophilin1

Cat.No. 159-0P; control peptide, 100 µg peptide (lyophilized)

Data Sheet

Reconstitution/ Storage	100 μg peptide, lyophilized. For reconstitution add 100 μl H ₂ O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Control peptides should be stored at -20°C when still lyophilized! For detailed information, see back of the data sheet.
Immunogen	Synthetic peptide corresponding to AA 256 to 276 from mouse Endophilin1 (UniProt Id: Q62420)
Recommended dilution	Optimal concentrations should be determined by the end-user.
Matching antibodies	159 002, 159 004
Remarks	This control peptide consists of the synthetic peptide (QPKPRMSLEFATGDSTQ) that has been used for immunization. It has been tested in preadsorption experiments and blocks efficiently and specifically the corresponding signal in Western blots. The amount of peptide needed for efficient blocking depends on the titer and on the affinity of the antibody to the antigen.

TO BE USED IN VITRO / FOR RESEARCH ONLY NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

Background

Endophilins are SH3 domain proteins involved in endocytosis. Both, **Endophilin 1** and **2** have been shown to play important roles in clathrin mediated synaptic vesicle recycling. They recruite and stabilize the polyphosphoinositide phosphatase synaptojanin at nerve terminals.

The divergent C-terminal tail of VgluT1 has been reported to be a binding partner of Endophilin A1. In contrast to Endophilin 1 that shows a brain specific expression, Endophilin 2 is abundantly expressed in different tissues.

Selected General References

Interaction between the vesicular glutamate transporter type 1 and endophilin A1, a protein essential for endocytosis. Vinatier J et al. J. Neurochem. (2006) PubMed:16606361

Endophilin is required for synaptic vesicle endocytosis by localizing synaptojanin.

Schuske KR et al. Neuron (2003) PubMed:14622579

 $\label{thm:continuous} Synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating.$

Verstreken P et al. Neuron (2003) PubMed:14622578

Endophilin and synaptojanin hook up to promote synaptic vesicle endocytosis.

Song W et al. Neuron (2003) PubMed:14622570

 $Formation \ of \ an \ end ophilin-Ca2+\ channel\ complex\ is\ critical\ for\ clathrin-mediated\ synaptic\ vesicle\ endocytosis.$

Chen Y et al. Cell (2003) PubMed:14532001

Endophilin-1: a multifunctional protein.

Reutens AT et al. Int. J. Biochem. Cell Biol. (2002) PubMed:12127567

Endophilin mutations block clathrin-mediated endocytosis but not neurotransmitter release.

Verstreken P et al. Cell (2002) PubMed:11955450

Differential expression of endophilin 1 and 2 dimers at central nervous system synapses.

Ringstad N et al. J. Biol. Chem. (2001) PubMed:11518713

Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of

Gad H et al. Neuron (2000) PubMed:10985350

Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis. Ringstad N et al. Neuron (1999) PubMed:10677033

Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid.

Schmidt A et al. Nature (1999) PubMed:10490020

Synaptojanin forms two separate complexes in the nerve terminal. Interactions with endophilin and amphiphysin.

Micheva KD et al. J. Biol. Chem. (1997) PubMed:9341169

SH3 domain-dependent interactions of endophilin with amphiphysin.

Micheva KD et al. FEBS Lett. (1997) PubMed:9315708

The SH3p4/Sh3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain

Ringstad N et al. Proc. Natl. Acad. Sci. U.S.A. (1997) PubMed:9238017

Access the online factsheet including applicable protocols at https://sysy.com/product/159-0P or scan the QR-code.



FAQ - How should I store my antibody?

Shipping Conditions

 All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freezedried) and are stable in this form without loss of quality at ambient temperatures for several weeks.

Storage of Sealed Vials after Delivery

- Unlabeled and biotin-labeled antibodies and control proteins should be stored at 4°C before reconstitution. They must not be stored in the freezer when still lyophilized!
 Temperatures below zero may cause loss of performance.
- Fluorescence-labeled antibodies should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle
 between freezing and thawing (to reduce frost-build-up), which is exactly what should be
 avoided. For the same reason, antibody vials should be placed in an area of the freezer that
 has minimal temperature fluctuations, for instance towards the back rather than on a door
 shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl)
 and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock
 concentration is affected by evaporation and adsorption of the antibody to the surface of the
 storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of
 activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

• Store at -20°C to -80°C.

Monoclonal Antibodies

- Ascites and hybridoma supernatant should be stored at -20°C up to -80°C. Prolonged storage at 4°C is not recommended! Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Polyclonal Antibodies

- Crude antisera: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- Affinity purified antibodies: Less robust than antisera. Storage at -20°C up to -80°C is
 recommended. Adding a carrier protein like BSA will increase long term stability. Most of our
 antibodies already contain carrier proteins. Please refer to the data-sheet for detailed
 information.

Fluorescence-labeled Antibodies

• Store as a liquid with 1:1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add
 the amount of deionized water given in the respective datasheet. If higher volumes are
 preferred, add water as mentioned above and then the desired amount of PBS and a
 stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies
 already contain albumin. Take this into account when adding more carrier protein.
 For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the
 solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled
 with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1:1 (v/v) glycerol to a final
 concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in
 liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.