SYSY Antibodies

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Endophilin2

Cat.No. 159 103; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For **reconstitution** add 50 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	**WB**: 1 : 1000 up to 1 : 5000 (AP staining) **IP**: not tested yet **ICC**: 1 : 500 **IHC**: 1 : 500 **IHC-P (FFPE)**: not tested yet
Immunogen	Synthetic peptide corresponding to AA 256 to 270 from mouse Endophilin2 (UniProt Id: Q62419)
Reactivity	Reacts with: mouse (Q62419), rat (O35964). Other species not tested yet.

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

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 endophilin-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 endophilin.
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/159103 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable 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. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.