

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 ₂ O 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: 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 recruit 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, Herzog E, Plamont MA, Wojcik SM, Schmidt A, Brose N, Daviet L, El Mestikawy S, Giros B *Journal of neurochemistry* (2006) 974: 1111-25. .

Endophilin is required for synaptic vesicle endocytosis by localizing synaptojanin. Schuske KR, Richmond JE, Matthies DS, Davis WS, Runz S, Rube DA, van der Bliek AM, Jorgensen EM *Neuron* (2003) 404: 749-62. .

Synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating. Verstreken P, Koh TW, Schulze KL, Zhai RG, Hiesinger PR, Zhou Y, Mehta SQ, Cao Y, Roos J, Bellen HJ *Neuron* (2003) 404: 733-48. .

Endophilin and synaptojanin hook up to promote synaptic vesicle endocytosis. Song W, Zinsmaier KE *Neuron* (2003) 404: 665-7. .

Formation of an endophilin-Ca²⁺ channel complex is critical for clathrin-mediated synaptic vesicle endocytosis. Chen Y, Deng L, Maeno-Hikichi Y, Lai M, Chang S, Chen G, Zhang JF *Cell* (2003) 1151: 37-48. .

Endophilin-1: a multifunctional protein. Reutens AT, Begley CG *The international journal of biochemistry & cell biology* (2002) 3410: 1173-7. .

Endophilin mutations block clathrin-mediated endocytosis but not neurotransmitter release. Verstreken P, Kjaerulff O, Lloyd TE, Atkinson R, Zhou Y, Meinertzhagen IA, Bellen HJ *Cell* (2002) 1091: 101-12. .

Differential expression of endophilin 1 and 2 dimers at central nervous system synapses. Ringstad N, Nemoto Y, De Camilli P *The Journal of biological chemistry* (2001) 27644: 40424-30. .

Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin. Gad H, Ringstad N, Löw P, Kjaerulff O, Gustafsson J, Wenk M, Di Paolo G, Nemoto Y, Crun J, Ellisman MH, De Camilli P, et al. *Neuron* (2000) 272: 301-12. .

Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis. Ringstad N, Gad H, Löw P, Di Paolo G, Brodin L, Shupliakov O, De Camilli P *Neuron* (1999) 241: 143-54. .

Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. Schmidt A, Wolde M, Thiele C, Fest W, Kratzin H, Podtelejnikov AV, Witke W, Huttner WB, Söling HD *Nature* (1999) 4016749: 133-41. .

Synaptojanin forms two separate complexes in the nerve terminal. Interactions with endophilin and amphiphysin. Micheva KD, Kay BK, McPherson PS *The Journal of biological chemistry* (1997) 27243: 27239-45. .

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 our antibodies and control proteins / peptides are shipped lyophilized (vacuum freeze-dried) 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 freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.