SYSY Antibodies

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Endobrevin (Vamp8)

Cat.No. 104 303; 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 (AP staining) **IP**: not tested yet **ICC**: 1 : 100 up to 1 : 500 **IHC**: external data (see remarks) **IHC-P (FFPE)**: not tested yet
Immunogen	Recombinant protein corresponding to AA 1 to 75 from rat Endobrevin (UniProt Id: Q9WUF4)
Reactivity	Reacts with: human (Q9BV40), rat (Q9WUF4), mouse (O70404). Other species not tested yet.
Specificity	K.O. validated PubMed: 25138214
Remarks	**IHC**: This antibody has been successfully applied and published for this method by customers (see application-specific references). It has not been validated using our standard protocols.

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

Background

Endobrevin/VAMP8, a member of the SNARE family of proteins, is a relative of synaptobrevin that is involved in the fusion of early and late endosomes. Endobrevin is expressed in most mammalian cells but appears to be absent from neurons. The protein is predominantly localized to early and late endosomal membranes but is also found on other membranes and organelles involved in endocytic membrane traffic. In the fusion of late endosomes it forms SNARE complexes with syntaxin 7, syntaxin 8 and vti1b.

Selected References for 104 303

Vesicle associated membrane protein 8 (VAMP8)-mediated zymogen granule exocytosis is dependent on endosomal trafficking via the constitutive-like secretory pathway.
Messenger SW, Falkowski MA, Thomas DD, Jones EK, Hong W, Gaisano HY, Giasano HY, Boulis NM, Groblewski GE
The Journal of biological chemistry (2014) 28940: 28040-53. . **WB, IHC; KO verified**

Plasma membrane flipping of Syntaxin-2 regulates its inhibitory action on insulin granule exocytosis.
Kang F, Xie L, Qin T, Miao Y, Kang Y, Takahashi T, Liang T, Xie H, Gaisano HY
Nature communications (2022) 131: 6512. . **IP, WB; tested species: human,mouse**

Defective AP-3-dependent VAMP8 trafficking impairs Weibel-Palade body exocytosis in Hermansky-Pudlak Syndrome type 2 blood outgrowth endothelial cells.
Karampini E, Schillemans M, Hofman M, van Alphen F, de Boer M, Kuijpers TW, van den Biggelaar M, Voorberg J, Bierings R
Haematologica (2019) : . . **WB, ICC; KD verified; tested species: human**

The endothelial diapedesis synapse regulates transcellular migration of human T lymphocytes in a CX3CL1- and SNAP23-dependent manner.
Schoppmeyer R, van Steen ACI, Kempers L, Timmerman AL, Nolte MA, Hombrink P, van Buul JD
Cell reports (2022) 383: 110243. . **WB; tested species: human**

Fluorescence Lifetime Imaging Microscopy reveals rerouting of SNARE trafficking driving dendritic cell activation.
Verboogen DRJ, González Mancha N, Ter Beest M, van den Bogaart G
eLife (2017) 6: . . **ICC; tested species: human**

Syntaxin 8 regulates platelet dense granule secretion, aggregation, and thrombus stability.
Golebiewska EM, Harper MT, Williams CM, Savage JS, Goggs R, Fischer von Mollard G, Poole AW
The Journal of biological chemistry (2015) 2903: 1536-45. . **WB**

Selected General References

Membrane fusion and exocytosis.
Jahn R et al. Annu. Rev. Biochem. (1999) PubMed:10872468

Seven novel mammalian SNARE proteins localize to distinct membrane compartments.
Advani RJ et al. J. Biol. Chem. (1998) PubMed:9553086

Endobrevin, a novel synaptobrevin/VAMP-like protein preferentially associated with the early endosome.
Wong SH et al. Mol. Biol. Cell (1998) PubMed:9614193

Access the online factsheet including applicable protocols at https://sysy.com/product/104303 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.