

VAMP1/2/3

Cat.No. 104 203; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

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Reconstitution/ Storage	50 μg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were 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) (see remarks) IP: not recommended ICC: 1 : 500 (see remarks) IHC: 1 : 200 IHC-P: 1 : 500 DNA-PAINT: yes (see remarks) ELISA: yes (see remarks)
Immunogen	Recombinant protein corresponding to AA 1 to 82 from rat Cellubrevin (UniProt Id: P63025)
Reactivity	Reacts with: human (P23763, P63027, Q15836), rat (Q63666, P63045, P63025), mouse (Q62442, P63044, P63024), zebrafish. Other species not tested yet.
Specificity	Specific for all three isoforms VAMP 1, 2 and 3. K.D. validated PubMed: <u>31128202</u>
Remarks	 WB: This antibody detects the tetanus and Botulinum B toxin cleavage product in toxin treated tissue homogenates. ICC: Methanol fixation is recommended. This antibody requires an additional acetone fixation step according to <u>Annaert</u> et al., 1997 This antibody is recommended for ICC of non-neuronal cells. DNA-PAINT: This antibody has been successfully used for DNA-PAINT application (see Unterauer et al., 2024; <u>PMID: 38552614</u>). ELISA: Suitable as detector antibody for sandwich-ELISA with cat. no. <u>104 211</u> as capture antibodies. The ELISA-protocol for membrane proteins is recommended.

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

Background

Synaptobrevins/VAMPs represents a family of integral membrane proteins of 11-13 kDa with the Nterminal region exposed to the cytoplasm and a C-terminal transmembrane domain. Two isoforms were identified in the mammalian CNS, synaptobrevin 1 (VAMP 1 or p18-1) and synaptobrevin 2 (VAMP 2 or p18-2) that differ in their distribution within different brain regions.

Synaptobrevin 1 is highly conserved between vertebrates and invertebrates. It is a major constituent of synaptic vesicles and peptidergic secretory granules in all neurons examined so far. In addition, it is present on secretory granules of neuroendocrine cells. Low levels of synaptobrevin 2 are present in many other tissues where the protein resides on specialized microvesicles.

In non-neuronal cells the third isoform, cellubrevin (VAMP 3), is present where it is localized to an endosomal membrane pool.

Synaptobrevin/VAMP is an essential component of the exocytotic fusion machine, related to a larger protein family referred to as v-SNAREs. It is the sole target for tetanus and several of the botulinal neurotoxins which cleave the protein at single sites in the C-terminal portion of the molecule.

Selected References for 104 203

Adaptor Protein-3-Mediated Trafficking of TLR2 Ligands Controls Specificity of Inflammatory Responses but Not Adaptor Complex Assembly. Petnicki-Ocwieja T, Kern A, Killpack TL, Bunnell SC, Hu LT

Journal of immunology (Baltimore, Md. : 1950) (2015) 1959: 4331-40. . **WB, ICC**

Role of SNAREs in the Atopic Dermatitis-related Cytokine Secretion and Skin-Nerve Communication. Meng J, Wang J, Buddenkotte J, Buhl T, Steinhoff M The Journal of investigative dermatology (2019) : . . **WB, ICC; KD verified; tested species: human**

Spatial proteomics in neurons at single-protein resolution. Unterauer EM, Shetab Boushehri S, Jevdokimenko K, Masullo LA, Ganji M, Sograte-Idrissi S, Kowalewski R, Strauss S, Reinhardt SCM, Perovic A, Marr C, et al. Cell (2024) 1877: 1785-1800.e16.. DNA_PAINT; tested species: rat

Generation of self-organized autonomic ganglion organoids from fibroblasts. Liu S, Xiang K, Yuan F, Xiang M iScience (2023) 263: 106241. . **ICC; tested species: mouse**

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**

TLR7 trafficking and signaling in B cells is regulated by the MHCII-associated invariant chain. Tohme M, Maisonneuve L, Achour K, Dussiot M, Maschalidi S, Manoury B Journal of cell science (2020) 1335: . . **ICC; tested species: mouse**

The Kohlschütter-Tönz syndrome associated gene Rogdi encodes a novel presynaptic protein. Riemann D, Wallrafen R, Dresbach T Scientific reports (2017) 71: 15791. . **ICC; tested species: rat**

Botulinum protease-cleaved SNARE fragments induce cytotoxicity in neuroblastoma cells. Arsenault J, Cuijpers SA, Ferrari E, Niranjan D, Rust A, Leese C, O'Brien JA, Binz T, Davletov B Journal of neurochemistry (2014) 1295: 781-91. . **ICC; tested species: mouse**

Access the online factsheet including applicable protocols at <u>https://sysy.com/product/104203</u> 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.