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# Synaptotagmin1 luminal domain

Cat.No. 105 103; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

## **Data Sheet**

Reconstitution/ Storage	50 $\mu g$ specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For <b>reconstitution</b> add 50 $\mu l$ H <sub>2</sub> 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 (AP staining)  IP: yes  ICC: 1: 100 up to 1: 500 (see remarks)  IHC: 1: 500 (rat only)  IHC_P: not tested yet
Immunogen	Synthetic peptide corresponding to AA 1 to 8 from mouse Synaptotagmin1 (UniProt Id: P46096)
Reactivity	Reacts with: rat (P21707), mouse (P46096). Other species not tested yet. For unknown reasons antibodies raised against the luminal N-terminus of Synaptotagmin 1 show a strong preference for the rat protein.
Specificity	K.O. PubMed: <u>31501440</u>
Matching control	105-10P
Remarks	<b>ICC</b> : This antibody is intended to be used for direct labeling of recycling synapses in primary neuronal cultures.

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

## Background

**Synaptotagmin 1** also known as **p65**, is an integral membrane glycoprotein of neuronal synaptic vesicles and secretory granules of neuroendocrine cells that is widely (but not ubiquitously) expressed in the central and peripheral nervous system. It has a variable N-terminal domain that is exposed to the lumen of the vesicle and a conserved cytoplasmic tail that contains two Ca<sup>2+</sup>-binding C2-domains. Ca<sup>2+</sup>-binding to synaptotagmin triggers exocytosis of synaptic vesicles, thus linking Ca<sup>2+</sup>-influx during depolarization to neurotransmitter release.

Lumenal antibodies were used in living neurons to label synaptic vesicles from the outside via endocytotic uptake.

#### Selected References for 105 103

Expression and secretion of synaptic proteins during stem cell differentiation to cortical neurons.

Nazir FH, Becker B, Brinkmalm A, Höglund K, Sandelius Å, Bergström P, Satir TM, Öhrfelt A, Blennow K, Agholme L, Zetterberg H, et al.

Neurochemistry international (2018):.. WB, ICC; tested species: human

Synaptophysin sustains presynaptic performance by preserving vesicular synaptobrevin-II levels.

Kokotos AC, Harper CB, Marland JRK, Smillie KJ, Cousin MA, Gordon SL Journal of neurochemistry (2019):.. WB, ICC; tested species: mouse

Control of exocytosis by synaptotagmins and otoferlin in auditory hair cells.

Beurg M, Michalski N, Safieddine S, Bouleau Y, Schneggenburger R, Chapman ER, Petit C, Dulon D

The Journal of neuroscience: the official journal of the Society for Neuroscience (2010) 3040: 13281-90. . IHC; tested species: rat

Phosphatidylinositol 4-kinase IIa is a glycogen synthase kinase 3-regulated interaction hub for activity-dependent bulk endocytosis.

Blumrich EM, Nicholson-Fish JC, Pronot M, Davenport EC, Kurian D, Cole A, Smillie KJ, Cousin MA Cell reports (2023) 426: 112633. . ICC; tested species: rat

Synaptotagmin-1-dependent phasic axonal dopamine release is dispensable for basic motor behaviors in mice.

Delignat-Lavaud B, Kano J, Ducrot C, Massé I, Mukherjee S, Giguère N, Moquin L, Lévesque C, Burke S, Denis R, Bourque MJ, et

bl.

Nature communications (2023) 141: 4120. . ICC; KO verified; tested species: mouse

Endosomal phosphatidylinositol 3-phosphate controls synaptic vesicle cycling and neurotransmission. Liu GT, Kochlamazashvili G, Puchkov D, Müller R, Schultz C, Mackintosh AI, Vollweiter D, Haucke V, Soykan T The EMBO journal (2022): e109352. . ICC; tested species: rat

Synaptotagmin 2 is ectopically overexpressed in excitatory presynapses of a widely used CaMKIIα-Cre mouse line.

Matsuura K, Mohamed HMA, Youssef MMM, Yoshida Y, Yamamoto T

iScience (2022) 258: 104692. . WB; tested species: mouse

Acute disruption of the synaptic vesicle membrane protein synaptotagmin 1 using knockoff in mouse hippocampal neurons. Vevea JD, Chapman ER

eLife (2020) 9: . . ICC; KO verified; tested species: mouse

Paroxysmal and cognitive phenotypes in Prrt2 mutant mice.

Robertson L, Featherby T, Howell S, Hughes J, Thomas P

Genes, brain, and behavior (2019): e12566. . WB; tested species: mouse

Synaptic neurexin-1 assembles into dynamically regulated active zone nanoclusters.

Trotter JH, Hao J, Maxeiner S, Tsetsenis T, Liu Z, Zhuang X, Südhof TC

The Journal of cell biology (2019) : . . ICC; tested species: mouse

Synaptotagmin 1 clamps synaptic vesicle fusion in mammalian neurons independent of complexin.

Courtney NA, Bao H, Briguglio JS, Chapman ER

Nature communications (2019) 101: 4076. . WB; KO verified; tested species: mouse

Access the online factsheet including applicable protocols at <a href="https://sysv.com/product/105103">https://sysv.com/product/105103</a> 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.