SYSY Antibodies

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Synaptotagmin1 (p65) luminal domain

Cat.No. 105 311AT647N; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized, fluorescence-labeled with ATTO® 647N. Albumin was added for stabilization. For **reconstitution** add 100 µl H_2O to get a 1mg/ml solution in PBS. Either add 1:1 (v/v) glycerol, then aliquot and store at -20°C until use, or store aliquots at -80°C without additives. Reconstitute immediately upon receipt! Avoid bright light when working with the antibody to minimize photo bleeching of the fluorescent dye. For detailed information, see back of the data sheet.
Applications	**WB**: N/A **IP**: N/A **ICC**: 1 : 50 up to 1 : 300 **IHC**: not tested yet **IHC-P**: not tested yet
Label	ATTO 647N
Clone	604.2
Subtype	IgG1 (κ light chain)
Immunogen	Synthetic peptide corresponding to residues near the amino terminus of rat Synaptotagmin1 (UniProt Id: P21707)
Reactivity	Reacts with: rat (P21707). No signal: mouse (P46096), zebrafish. Other species not tested yet.
Remarks	**ICC**: This antibody can also be used for labeling of recycling synaptic vesicles in living neurons.

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

Background

Synaptotagmin1, 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^{2+}-binding C2-domains. Ca^{2+}-binding to synaptotagmin triggers exocytosis of synaptic vesicles, thus linking Ca^{2+}-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 311AT647N

Newly produced synaptic vesicle proteins are preferentially used in synaptic transmission.
Truckenbrodt S, Viplav A, Jähne S, Vogts A, Denker A, Wildhagen H, Fornasiero EF, Rizzoli SO
The EMBO journal (2018) : . . **ICC, UPTAKE; tested species: rat**

Neurofilament Levels in Dendritic Spines Associate with Synaptic Status.
Gürth CM, do Rego Barros Fernandes Lima MA, Macarrón Palacios V, Cereceda Delgado AR, Hubrich J, D'Este E
Cells (2023) 126: . . **UPTAKE; tested species: rat**

Presynaptic activity and protein turnover are correlated at the single-synapse level.
Jähne S, Mikulasch F, Heuer HGH, Truckenbrodt S, Agüi-Gonzalez P, Grewe K, Vogts A, Rizzoli SO, Priesemann V
Cell reports (2021) 3411: 108841. . **ICC; tested species: rat**

Synaptic activity and strength are reflected by changes in the post-synaptic secretory pathway.
Gürth CM, Dankovich TM, Rizzoli SO, D'Este E
Scientific reports (2020) 101: 20576. . **UPTAKE; tested species: rat**

Rho-kinase inhibition by fasudil modulates pre-synaptic vesicle dynamics.
Saal KA, Warth Pérez Arias C, Roser AE, Christoph Koch J, Bähr M, Rizzoli SO, Lingor P
Journal of neurochemistry (2020) : . . **UPTAKE; tested species: rat**

STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons.
D'Este E, Kamin D, Göttfert F, El-Hady A, Hell SW
Cell reports (2015) 108: 1246-51. . **UPTAKE; tested species: rat**

Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins.
Wilhelm BG, Mandad S, Truckenbrodt S, Kröhnert K, Schäfer C, Rammner B, Koo SJ, Claßen GA, Krauss M, Haucke V, Urlaub H, et al.
Science (New York, N.Y.) (2014) 3446187: 1023-8. . **ICC; tested species: rat**

Blocking endocytosis enhances short-term synaptic depression under conditions of normal availability of vesicles.
Hua Y, Woehler A, Kahms M, Haucke V, Neher E, Klingauf J
Neuron (2013) 802: 343-9. . **ICC; tested species: rat**

The same synaptic vesicles drive active and spontaneous release.
Wilhelm BG, Groemer TW, Rizzoli SO
Nature neuroscience (2010) 1312: 1454-6. . **UPTAKE**

Limited intermixing of synaptic vesicle components upon vesicle recycling.
Opazo F, Punge A, Bückers J, Hoopmann P, Kastrup L, Hell SW, Rizzoli SO
Traffic (Copenhagen, Denmark) (2010) 116: 800-12. . **ICC**

Selected General References

RAB3 and synaptotagmin: the yin and yang of synaptic membrane fusion.
Geppert M et al. Annu. Rev. Neurosci. (1998) PubMed:9530492

Access the online factsheet including applicable protocols at https://sysy.com/product/105311AT647N 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.