SYSY Antibodies

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Synaptotagmin1 (p65) luminal domain

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

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized, fluorescence-labeled with CypHer5E. 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 (see remarks) **IHC**: not tested yet **IHC-P**: not tested yet **EM**: N/A **ELISA**: N/A **FACS**: not tested yet
Label	CypHer5E
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 only be used for labeling of recycling synaptic vesicles in living neurons. It is not recommended for the staining of fixed cells. The pH sensitive dye regaines its fluorescence after the reacidification of the synaptic vesicle lumen.

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 311CpH

Key physiological parameters dictate triggering of activity-dependent bulk endocytosis in hippocampal synapses.
Wenzel EM, Morton A, Ebert K, Welzel O, Kornhuber J, Cousin MA, Groemer TW
PloS one (2012) 76: e38188. . **UPTAKE**

Synapse clusters are preferentially formed by synapses with large recycling pool sizes.
Welzel O, Tischbirek CH, Jung J, Kohler EM, Svetlitchny A, Henkel AW, Kornhuber J, Groemer TW
PloS one (2010) 510: e13514. . **ICC**

α-Synuclein induced cholesterol lowering increases tonic and reduces depolarization-evoked synaptic vesicle recycling and glutamate release.
Lazarevic V, Yang Y, Paslawski W, Svenningsson P
NPJ Parkinson's disease (2022) 81: 71. . **UPTAKE; tested species: mouse**

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**

CtBP1-Mediated Membrane Fission Contributes to Effective Recycling of Synaptic Vesicles.
Ivanova D, Imig C, Camacho M, Reinhold A, Guhathakurta D, Montenegro-Venegas C, Cousin MA, Gundelfinger ED, Rosenmund C, Cooper B, Fejtova A, et al.
Cell reports (2020) 307: 2444-2459.e7. . **UPTAKE; tested species: mouse**

Transient Confinement of CaV2.1 Ca2+-Channel Splice Variants Shapes Synaptic Short-Term Plasticity.
Heck J, Parutto P, Ciuraszkiewicz A, Bikbaev A, Freund R, Mitlöhner J, Alonso M, Fejtova A, Holcman D, Heine M
Neuron (2019) : . . **ICC; tested species: rat**

Riluzole attenuates the efficacy of glutamatergic transmission by interfering with the size of the readily releasable neurotransmitter pool.
Lazarevic V, Yang Y, Ivanova D, Fejtova A, Svenningsson P
Neuropharmacology (2018) : . . **ICC; tested species: rat**

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) : . . **UPTAKE; tested species: rat**

Regulated Dynamic Trafficking of Neurexins Inside and Outside of Synaptic Terminals.
Neupert C, Schneider R, Klatt O, Reissner C, Repetto D, Biermann B, Niesmann K, Missler M, Heine M
The Journal of neuroscience : the official journal of the Society for Neuroscience (2015) 3540: 13629-47. . **ICC**

Dynamic properties of the alkaline vesicle population at hippocampal synapses.
Röther M, Brauner JM, Ebert K, Welzel O, Jung J, Bauereiss A, Kornhuber J, Groemer TW
PloS one (2014) 97: e102723. . **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. . **UPTAKE; tested species: rat**

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