SYSY Antibodies

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VGAT (SLC32A1) luminal domain

Cat.No. 131 103CpH; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen, fluorescence-labeled with CypHer5E. Albumin was added for stabilization. For **reconstitution** add 50 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 : 100 up to 1 : 200 (see remarks) **IHC**: not tested yet **IHC-P (FFPE)**: not tested yet
Label	CypHer5E
Immunogen	Synthetic peptide corresponding to residues near the carboxy terminus of rat VGAT (UniProt Id: O35458)
Reactivity	Reacts with: human (Q9H598), rat (O35458), mouse (O35633). Other species not tested yet.
Specificity	K.O. validated
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

The **v**esicular **GA**BA **t**ransporter **VGAT** is responsible for uptake and storage of GABA and glycine by synaptic vesicles in the central nervous system. For this reason it is frequently referred to as the **v**esicular **i**nhibitory **a**mino**a**cid **t**ransporter **VIAAT**. It is different from the plasma membrane transporters in that it is driven by a proton electrochemical gradient across the vesicle membrane. So far, only one isoform is known. VGAT is currently the best marker for inhibitory nerve terminals.

Selected References for 131 103CpH

A readily retrievable pool of synaptic vesicles.
Hua Y, Sinha R, Thiel CS, Schmidt R, Hüve J, Martens H, Hell SW, Egner A, Klingauf J
Nature neuroscience (2011) 147: 833-9. . **UPTAKE**

Chronic benzodiazepine treatment triggers gephyrin scaffold destabilization and GABAAR subsynaptic reorganization.
Chapman CA, Povysheva N, Tarr TB, Nuwer JL, Meriney SD, Johnson JW, Jacob TC
Frontiers in cellular neuroscience (2025) 19: 1624813. . **UPTAKE; tested species: rat**

Rho GTPase signaling and mDia facilitate endocytosis via presynaptic actin.
Oevel K, Hohensee S, Kumar A, Rosas-Brugada I, Bartolini F, Soykan T, Haucke V
eLife (2024) 12: . . **UPTAKE; tested species: mouse**

Clathrin-independent endocytic retrieval of SV proteins mediated by the clathrin adaptor AP-2 at mammalian central synapses.
López-Hernández T, Takenaka KI, Mori Y, Kongpracha P, Nagamori S, Haucke V, Takamori S
eLife (2022) 11: . . **UPTAKE; tested species: mouse**

Unique dynamics and exocytosis properties of GABAergic synaptic vesicles revealed by three-dimensional single vesicle tracking.
Park C, Chen X, Tian CL, Park GN, Chenouard N, Lee H, Yeo XY, Jung S, Tsien RW, Bi GQ, Park H, et al.
Proceedings of the National Academy of Sciences of the United States of America (2021) 1189: . . **UPTAKE; 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**

Unique luminal localization of VGAT-C terminus allows for selective labeling of active cortical GABAergic synapses.
Martens H, Weston MC, Boulland JL, Grønborg M, Grosche J, Kacza J, Hoffmann A, Matteoli M, Takamori S, Harkany T, Chaudhry FA, et al.
The Journal of neuroscience : the official journal of the Society for Neuroscience (2008) 2849: 13125-31. . **UPTAKE; tested species: rat**

A common origin of synaptic vesicles undergoing evoked and spontaneous fusion.
Hua Y, Sinha R, Martineau M, Kahms M, Klingauf J
Nature neuroscience (2010) 1312: 1451-3. .

Selected General References

The vesicular GABA transporter, VGAT, localizes to synaptic vesicles in sets of glycinergic as well as GABAergic neurons.
Chaudhry FA et al. J. Neurosci. (1998) PubMed:9822734

Identification and characterization of the vesicular GABA transporter.
McIntire SL et al. Nature (1997) PubMed:9349821

Cloning of a functional vesicular GABA and glycine transporter by screening of genome databases.
Sagné C et al. FEBS Lett. (1997) PubMed:9395291

Uptake of GABA by rat brain synaptic vesicles isolated by a new procedure.
Hell JW et al. EMBO J. (1988) PubMed:2903047

Access the online factsheet including applicable protocols at https://sysy.com/product/131103CpH 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.