

VGAT 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 ₂ O 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 bleaching 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: 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 is intended for direct labeling of recycling synaptic vesicles at inhibitory nerve terminals in living primary neuronal cultures. The pH sensitive dye regains its fluorescence after the reacidification of the synaptic vesicle lumen. Further details see Martens et al. 2008.

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

Background

The vesicular **GABA** transporter **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 vesicular inhibitory aminoacid transporter **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**
- 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 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.