

VGAT luminal domain

Cat.No. 131 103C2; 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 Cyanine 2. 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: yes IHC-P: not tested yet
Label	Cyanine 2
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 labeling of recycling synaptic vesicles at inhibitory nerve terminals by adding to living neurons. 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 amino acid 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 103C2

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: . . **ICC; tested species: mouse**

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**

A ratiometric ER calcium sensor for quantitative comparisons across cell types and subcellular regions. Farrell RJ, Bredvik KG, Hoppa MB, Hennigan ST, Brown TA, Ryan TA bioRxiv : the preprint server for biology (2024) : . . **UPTAKE; tested species: rat**

Stabilization of GABA(A) receptors at endocytic zones is mediated by an AP2 binding motif within the GABA(A) receptor β3 subunit.

Smith KR, Muir J, Rao Y, Browarski M, Gruenig MC, Sheehan DF, Haucke V, Kittler JT The Journal of neuroscience : the official journal of the Society for Neuroscience (2012) 327: 2485-98. . **UPTAKE**

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/131103C2> 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.