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# **VGAT** luminal domain

Cat.No. 131 103C5; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

## **Data Sheet**

Reconstitution/ Storage	50 $\mu g$ specific antibody, lyophilized. Affinity purified with the immunogen, fluorescence-labeled with Cyanine 5. Albumin was added for stabilization. For <b>reconstitution</b> add 50 $\mu$ l H <sub>2</sub> 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 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: not tested yet
Label	Sulfo-Cyanine 5
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	<b>ICC</b> : This antibody can also be used for <u>labeling of recycling synaptic vesicles</u> in living neurons. Further details see <u>Martens</u> 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 103C5

Mechanism and effects of pulsatile GABA secretion from cytosolic pools in the human beta cell.

Menegaz D, Hagan DW, Almaça J, Cianciaruso C, Rodriguez-Diaz R, Molina J, Dolan RM, Becker MW, Schwalie PC, Nano R, Lebreton F. et al.

Nature metabolism (2019) 111: 1110-1126. . ICC, IHC; tested species: human,rat

miRNA-mediated control of gephyrin synthesis drives sustained inhibitory synaptic plasticity.

Welle TM, Rajgor D, Kareemo DJ, Garcia JD, Zych SM, Wolfe SE, Gookin SE, Martinez TP, Dell'Acqua ML, Ford CP, Kennedy MJ, et al.

EMBO reports (2024) 2511: 5141-5168. . UPTAKE; tested species: rat

Microglial Displacement of GABAergic Synapses Is a Protective Event during Complex Febrile Seizures.

Wan Y, Feng B, You Y, Yu J, Xu C, Dai H, Trapp BD, Shi P, Chen Z, Hu W

Cell reports (2020) 335: 108346. . IHC; tested species: mouse

Inter-Synaptic Lateral Diffusion of GABAA Receptors Shapes Inhibitory Synaptic Currents.

de Luca E, Ravasenga T, Petrini EM, Polenghi A, Nieus T, Guazzi S, Barberis A

Neuron (2017) 951: 63-69.e5. . ICC; tested species: mouse

Nanoscale Molecular Reorganization of the Inhibitory Postsynaptic Density Is a Determinant of GABAergic Synaptic Potentiation.

Pennacchietti F, Vascon S, Nieus T, Rosillo C, Das S, Tyagarajan SK, Diaspro A, Del Bue A, Petrini EM, Barberis A, Cella Zanacchi F, et al.

The Journal of neuroscience: the official journal of the Society for Neuroscience (2017) 377: 1747-1756. . ICC

Synaptic recruitment of gephyrin regulates surface GABAA receptor dynamics for the expression of inhibitory LTP. Petrini EM, Ravasenga T, Hausrat TJ, Iurilli G, Olcese U, Racine V, Sibarita JB, Jacob TC, Moss SJ, Benfenati F, Medini P, et al. Nature communications (2014) 5: 3921. ICC; tested species: mouse

#### **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 <a href="https://sysy.com/product/131103C5">https://sysy.com/product/131103C5</a> 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 freezedried) 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 freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.