SYSY Antibodies

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VGAT luminal domain

Cat.No. 131 103C5; 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 5. 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**: yes **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.
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 **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 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**

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**

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**

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**

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, Reimer RJ, Bellocchio EE, Danbolt NC, Osen KK, Edwards RH, Storm-Mathisen J
The Journal of neuroscience : the official journal of the Society for Neuroscience (1998) 1823: 9733-50. .

Identification and characterization of the vesicular GABA transporter.
McIntire SL, Reimer RJ, Schuske K, Edwards RH, Jorgensen EM
Nature (1997) 3896653: 870-6. .

Cloning of a functional vesicular GABA and glycine transporter by screening of genome databases.
Sagné C, El Mestikawy S, Isambert MF, Hamon M, Henry JP, Giros B, Gasnier B
FEBS letters (1997) 4172: 177-83. .

Uptake of GABA by rat brain synaptic vesicles isolated by a new procedure.
Hell JW, Maycox PR, Stadler H, Jahn R
The EMBO journal (1988) 710: 3023-9. .

Access the online factsheet including applicable protocols at https://sysy.com/product/131103C5 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.