SYSY Antibodies

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VGAT cytoplasmic domain

Cat.No. 131 005; Polyclonal Guinea pig antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For **reconstitution** add 50 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	**WB**: 1 : 1000 up to 1 : 5000 **IP**: not tested yet **ICC**: 1 : 500 up to 1 : 1000 **IHC**: 1 : 500 up to 1 : 1000 (see remarks) **IHC-P**: 1 : 500
Immunogen	Recombinant protein corresponding to residues near the amino terminus of rat VGAT (UniProt Id: O35458)
Reactivity	Reacts with: rat (O35458), mouse (O35633). Other species not tested yet.
Matching control	131-0GP
Remarks	VGAT aggregates after boiling, making it necessary to run SDS-PAGE only with non-boiled samples. **IHC**: Signal intensity can be enhanced by antigen retrieval at pH6 prior to blocking step.

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 131 005

Identification and characterization of GABA(A) receptor autoantibodies in autoimmune encephalitis.
Ohkawa T, Satake S, Yokoi N, Miyazaki Y, Ohshita T, Sobue G, Takashima H, Watanabe O, Fukata Y, Fukata M
The Journal of neuroscience : the official journal of the Society for Neuroscience (2014) 3424: 8151-63. . **ICC; tested species: rat**

Corelease of dopamine and GABA by a retinal dopaminergic neuron.
Hirasawa H, Betensky RA, Raviola E
The Journal of neuroscience : the official journal of the Society for Neuroscience (2012) 3238: 13281-91. . **IHC; tested species: mouse**

GABAergic-like dopamine synapses in the brain.
Kim HJ, Hwang B, Reva M, Lee J, Lee BE, Lee Y, Cho EJ, Jeong M, Lee SE, Myung K, Baik JH, et al.
Cell reports (2023) 4210: 113239. . **IHC; tested species: mouse**

Engram cell connectivity as a mechanism for information encoding and memory function.
Ortega-de San Luis C, Pezzoli M, Urrieta E, Ryan TJ
Current biology : CB (2023) 3324: 5368-5380.e5. . **IHC; tested species: mouse**

Deleting Mecp2 from the cerebellum rather than its neuronal subtypes causes a delay in motor learning in mice.
Achilly NP, He LJ, Kim OA, Ohmae S, Wojaczynski GJ, Lin T, Sillitoe RV, Medina JF, Zoghbi HY
eLife (2021) 10: . . **IHC; tested species: mouse**

Direct reprogramming of oligodendrocyte precursor cells into GABAergic inhibitory neurons by a single homeodomain transcription factor Dlx2.
Boshans LL, Soh H, Wood WM, Nolan TM, Mandoiu II, Yanagawa Y, Tzingounis AV, Nishiyama A
Scientific reports (2021) 111: 3552. . **ICC; tested species: mouse**

Molecular self-avoidance in synaptic neurexin complexes.
Wang CY, Trotter JH, Liakath-Ali K, Lee SJ, Liu X, Südhof TC
Science advances (2021) 751: eabk1924. . **IHC; tested species: mouse**

Neuroligin-4 Regulates Excitatory Synaptic Transmission in Human Neurons.
Marro SG, Chanda S, Yang N, Janas JA, Valperga G, Trotter J, Zhou B, Merrill S, Yousif I, Shelby H, Vogel H, et al.
Neuron (2019) 1034: 617-626.e6. . **ICC; tested species: human**

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. .

Access the online factsheet including applicable protocols at https://sysy.com/product/131005 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.