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# **VGAT** cytoplasmic domain

Cat.No. 131 004; Polyclonal Guinea pig antibody, 100 µl antiserum (lyophilized)

#### **Data Sheet**

Reconstitution/ Storage	100 $\mu$ l antiserum, lyophilized. For <b>reconstitution</b> add 100 $\mu$ l H <sub>2</sub> O, then aliquot and store at -20°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: 2000 (AP staining)  IP: not tested yet  ICC: 1: 500 up to 1: 1000  IHC: 1: 200 up to 1: 500  IHC_P: yes  IHC_FR: 1: 500 (see remarks)  EXM: 1: 250 (see remarks)  EM: yes
Immunogen	Recombinant protein corresponding to residues near the amino terminus of rat VGAT (UniProt Id: O35458)
Reactivity	Reacts with: rat (O35458), mouse (O35633), zebrafish, ape. Other species not tested yet.
Specificity	K.O.
Matching control	131-0GP
Remarks	VGAT aggregates after boiling, making it necessary to run SDS-PAGE only with non-boiled samples.  IHC_FR: 5 min MeOH and PFA fixation are possible.  EXM: This antibody has been successfully used for the magnified analysis of the proteome (MAP) expansion microscopy method (MAP; Ku et al. 2016. Nature Biotechnology 34:973-981)

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 131 004

Quantitative comparison of glutamatergic and GABAergic synaptic vesicles unveils selectivity for few proteins including MAL2, a novel synaptic vesicle protein.

Grønborg M, Pavlos NJ, Brunk I, Chua JJ, Münster-Wandowski A, Riedel D, Ahnert-Hilger G, Urlaub H, Jahn R The Journal of neuroscience: the official journal of the Society for Neuroscience (2010) 301: 2-12. . ICC, IHC, EM

Colocalization of different neurotransmitter transporters on synaptic vesicles is sparse except for VGLUT1 and ZnT3. Upmanyu N, Jin J, Emde HV, Ganzella M, Bösche L, Malviya VN, Zhuleku E, Politi AZ, Ninov M, Silbern I, Leutenegger M, et al. Neuron (2022):.. WB, UPTAKE; tested species: rat

Development of dissociated cryopreserved rat cortical neurons in vitro.

Schock SC, Jolin-Dahel KS, Schock PC, Theiss S, Arbuthnott GW, Garcia-Munoz M, Staines WA

Journal of neuroscience methods (2012) 2052: 324-33. . WB, IHC

Spectrin-beta 2 facilitates the selective accumulation of GABAA receptors at somatodendritic synapses.

Smalley JL, Cho N, Ng SFJ, Choi C, Lemons AHS, Chaudry S, Bope CE, Dengler JS, Zhang C, Rasband MN, Davies PA, et al. Communications biology (2023) 61: 11. . WB, ICC; tested species: mouse

Microglia enable cross-modal plasticity by removing inhibitory synapses.

Hashimoto A, Kawamura N, Tarusawa E, Takeda I, Aoyama Y, Ohno N, Inoue M, Kagamiuchi M, Kato D, Matsumoto M, Hasegawa Y, et al.

Cell reports (2023) 425: 112383. . WB, IHC; tested species: mouse

Brevican, Neurocan, Tenascin-C, and Tenascin-R Act as Important Regulators of the Interplay Between Perineuronal Nets, Synaptic Integrity, Inhibitory Interneurons, and Otx2.

Mueller-Buehl C. Reinhard J. Roll L. Bader V. Winklhofer KF. Faissner A

Frontiers in cell and developmental biology (2022) 10: 886527. . WB, IHC; tested species: mouse

Targeted proteoform mapping uncovers specific Neurexin-3 variants required for dendritic inhibition.

Hauser D, Behr K, Konno K, Schreiner D, Schmidt A, Watanabe M, Bischofberger J, Scheiffele P

Neuron (2022) 11013: 2094-2109.e10. . ICC, IHC; tested species: mouse

Systemic inflammation induced the delayed reduction of excitatory synapses in the CA3 during ageing.

Manabe T, Rácz I, Schwartz S, Oberle L, Santarelli F, Emmrich JV, Neher JJ, Heneka MT

Journal of neurochemistry (2021) 1593: 525-542.. WB, IHC; tested species: mouse

Chemico-genetic discovery of astrocytic control of inhibition in vivo.

Takano T, Wallace JT, Baldwin KT, Purkey AM, Uezu A, Courtland JL, Soderblom EJ, Shimogori T, Maness PF, Eroglu C, Soderling SH, et al.

Nature (2020):.. ICC, IHC; tested species: mouse

Expression of Neurofilament Subunits at Neocortical Glutamatergic and GABAergic Synapses.

Bragina L. Conti F

Frontiers in neuroanatomy (2018) 12: 74. . WB, IHC; tested species: rat

The a3 subunit of GABAA receptors promotes formation of inhibitory synapses in the absence of collybistin.

Wagner S, Lee C, Rojas L, Specht CG, Rhee J, Brose N, Papadopoulos T

The Journal of biological chemistry () 296: 100709. . ICC, IHC; tested species: mouse  $\,$ 

Generation of mouse hippocampal brain organoids from primary embryonic neural stem cells.

Ciarpella F, Zamfir RG, Campanelli A, Pedrotti G, Di Chio M, Bottani E, Decimo I

STAR protocols (2023) 43: 102413. . IHC\_FR; tested species: mouse

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/131004">https://sysy.com/product/131004</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.