

## GABA-A receptor $\alpha 5$ extracellular

Cat.No. 224 503; Polyclonal rabbit antibody, 50  $\mu$ g specific antibody (lyophilized)

### Data Sheet

Reconstitution/ Storage	50 $\mu$ g specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For <b>reconstitution</b> add 50 $\mu$ l H <sub>2</sub> O 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	<b>WB:</b> 1 : 1000 (AP staining) (see remarks) <b>IP:</b> yes <b>ICC:</b> 1 : 500 <b>IHC:</b> 1 : 500 <b>IHC-P:</b> not tested yet
Immunogen	Synthetic peptide corresponding to AA 26 to 43 from rat GABA-A receptor $\alpha 5$ (UniProt Id: P19969)
Reactivity	Reacts with: rat (P19969), mouse (Q8BHJ7). Other species not tested yet.
Specificity	K.O. validated PubMed: <a href="https://pubmed.ncbi.nlm.nih.gov/27792253/">27792253</a>
Matching control	224-5P
Remarks	<b>WB:</b> GABA-A receptor alpha5 aggregates after boiling, making it necessary to run SDS-PAGE with non-boiled samples.

**TO BE USED IN VITRO / FOR RESEARCH ONLY**  
**NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS**

### Background

Gamma-aminobutyric acid type **A (GABA-A)** receptors mediate the majority of inhibitory neurotransmission in the brain. These receptor proteins are ligand gated chloride ion channels and consist of a pentameric combination of different subunits (**alpha**, beta, gamma, delta, epsilon and rho). The resulting heterogenous population of GABA-A receptor subtypes are expressed throughout the brain with specific cellular and subcellular expression patterns.

### Selected References for 224 503

- Synaptic localization of  $\alpha 5$  GABA (A) receptors via gephyrin interaction regulates dendritic outgrowth and spine maturation. Brady ML, Jacob TC  
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- Activity- and sleep-dependent regulation of tonic inhibition by Shisa7. Wu K, Han W, Tian Q, Li Y, Lu W  
Cell reports (2021) 3412: 108899. . **WB, ICC, UPTAKE; tested species: mouse**
- Distinct regulation of tonic GABAergic inhibition by NMDA receptor subtypes. Wu K, Castellano D, Tian Q, Lu W  
Cell reports (2021) 376: 109960. . **WB, UPTAKE; tested species: mouse**
- Sustained treatment with an  $\alpha 5$  GABA A receptor negative allosteric modulator delays excitatory circuit development while maintaining GABAergic neurotransmission. Nuwer JL, Brady ML, Povysheva NV, Coyne A, Jacob TC  
Neuropharmacology (2021) 197: 108724. . **WB, ICC; tested species: rat**
- $\alpha 5$ GABAA Receptors Mediate Tonic Inhibition in the Spinal Cord Dorsal Horn and Contribute to the Resolution Of Hyperalgesia. Perez-Sanchez J, Lorenzo LE, Lecker I, Zurek AA, Labrakakis C, Bridgwater EM, Orser BA, De Koninck Y, Bonin RP  
Journal of neuroscience research (2017) 956: 1307-1318. . **IHC; KO verified; tested species: mouse**
- Long-term  $\alpha 5$  GABA A receptor negative allosteric modulator treatment reduces NMDAR-mediated neuronal excitation and maintains basal neuronal inhibition. Nuwer JL, Povysheva N, Jacob TC  
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- Inhibitory and excitatory synaptic neuroadaptations in the diazepam tolerant brain. Lorenz-Guertin JM, Povysheva N, Chapman CA, MacDonald ML, Fazzari M, Nigam A, Nuwer JL, Das S, Brady ML, Vajn K, Bambino MJ, et al.  
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- Interaction between Teneurin-2 and microtubules via EB proteins provides a platform for GABAA receptor exocytosis. Ichinose S, Susuki Y, Hosoi N, Kaneko R, Ebihara M, Hirai H, Iwasaki H  
eLife (2023) 12: . . **ICC; tested species: mouse**
- FMRP regulates GABAA receptor channel activity to control signal integration in hippocampal granule cells. Deng PY, Kumar A, Cavalli V, Klyachko VA  
Cell reports (2022) 397: 110820. . **IP; tested species: mouse**
- Sleep and wake cycles dynamically modulate hippocampal inhibitory synaptic plasticity. Wu K, Han W, Lu W  
PLoS biology (2022) 2011: e3001812. . **WB; tested species: mouse**
- Sparification of AP firing in adult-born hippocampal granule cells via voltage-dependent  $\alpha 5$ -GABAA receptors. Lodge M, Hernandez MC, Schulz JM, Bischofberger J  
Cell reports (2021) 371: 109768. . **IHC; tested species: mouse**
- Enhancing neuronal chloride extrusion rescues  $\alpha 2/\alpha 3$  GABAA-mediated analgesia in neuropathic pain. Lorenzo LE, Godin AG, Ferrini F, Bachand K, Plasencia-Fernandez I, Labrecque S, Girard AA, Boudreau D, Kianicka I, Gagnon M, Doyon N, et al.  
Nature communications (2020) 111: 869. . **IHC; tested species: rat**

Access the online factsheet including applicable protocols at <https://sysy.com/product/224503> 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.