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# **Piccolo**

Cat.No. 142 104; 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	<b>WB</b> : 1: 1000 (AP staining) (see remarks) <b>IP</b> : yes <b>ICC</b> : 1: 500 up to 1: 1000 <b>IHC</b> : 1: 200 <b>IHC_P</b> : 1: 500
Immunogen	Recombinant protein corresponding to a central region of rat piccolo (UniProt Id: Q9JKS6)
Reactivity	Reacts with: rat (Q9JKS6), mouse (Q9QYX7). Other species not tested yet.
Specificity	K.O. PubMed: <u>32122952</u>
Remarks	<b>WB</b> : Due to its large size, piccolo requires special gel-electrophoresis and Western blot protocols for visualization by immunoblotting. Excellent results can be obtained with the 4-12% TRIS-glycine gradient gels from anamed or NuPAGE 3-8% TRIS-Acetate gradient gels from invitrogen.
	This antibody detects an additional band of ~65 kDa.

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

#### **Background**

**Piccolo**, also referred to as **Aczonin**, is a large protein which consists of an N-terminal Zn<sup>2+</sup> finger, several piccolo-bassoon homology domains (PBH-domains) and C-terminal PDZ and C2 domains. In general it is found together with bassoon, a related huge multi-domain protein of the CAZ (cytoskeletal matrix assembled at active zones).

Piccolo is supposed to be a scaffolding protein for proteins involved in endo- and exocytosis of synaptic vesicles. Recently piccolo has been shown to interfere with clathrin mediated endocytosis by binding to the F-actin and dynamin binding protein Abp1.

#### Selected References for 142 104

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) : . . ICC. UPTAKE: tested species: rat

HIV Tat causes synapse loss in a mouse model of HIV-associated neurocognitive disorder that is independent of the classical complement cascade component C1q.

Hammond JW, Qiu WQ, Marker DF, Chamberlain JM, Greaves-Tunnell W, Bellizzi MJ, Lu SM, Gelbard HA

Glia (2018):.. IHC; tested species: mouse

Synaptotagmin-3 drives AMPA receptor endocytosis, depression of synapse strength, and forgetting.

Awasthi A, Ramachandran B, Ahmed S, Benito E, Shinoda Y, Nitzan N, Heukamp A, Rannio S, Martens H, Barth J, Burk K, et al. Science (New York, N.Y.) (2018):.. WB; tested species: rat

URMC-099 prophylaxis prevents hippocampal vascular vulnerability and synaptic damage in an orthopedic model of delirium superimposed on dementia.

Miller-Rhodes P, Li H, Velagapudi R, Chiang W, Terrando N, Gelbard HA

FASEB journal: official publication of the Federation of American Societies for Experimental Biology (2022) 366: e22343.. IHC; tested species: mouse

Loss of FEZ1, a gene deleted in Jacobsen syndrome, causes locomotion defects and early mortality by impairing motor neuron development.

Gunaseelan S, Wang Z, Tong VKJ, Ming SWS, Razar RBBA, Srimasorn S, Ong WY, Lim KL, Chua JJE Human molecular genetics (2021):..ICC; tested species: human

Loss of miR-183/96 alters synaptic strength via pre- and postsynaptic mechanisms at a central synapse. Krohs C, Körber C, Ebbers L, Altaf F, Hollje G, Hoppe S, Dörflinger Y, Prosser HM, Nothwang HG

The Journal of neuroscience: the official journal of the Society for Neuroscience (2021):.. **IHC; tested species: mouse** 

Excitatory amino acid transporter EAAT5 improves temporal resolution in the retina.

Gehlen J, Aretzweiler C, Mataruga A, Fahlke C, Müller F

eNeuro (2021):.. IHC; tested species: mouse

A High-Resolution Method for Quantitative Molecular Analysis of Functionally Characterized Individual Synapses. Holderith N, Heredi J, Kis V, Nusser Z

Cell reports (2020) 324: 107968. . IHC; tested species: rat

Loss of Piccolo Function in Rats Induces Cerebellar Network Dysfunction and Pontocerebellar Hypoplasia Type 3-like Phenotypes.

Falck J, Bruns C, Hoffmann-Conaway S, Straub I, Plautz EJ, Orlando M, Munawar H, Rivalan M, Winter Y, Izsvák Z, Schmitz D, et al. The Journal of neuroscience: the official journal of the Society for Neuroscience (2020) 4014: 2943-2959. IHC; KO verified; tested species: rat

Kinesin Family of Proteins Kif11 and Kif21B Act as Inhibitory Constraints of Excitatory Synaptic Transmission Through Distinct Mechanisms.

Swarnkar S, Avchalumov Y, Raveendra BL, Grinman E, Puthanveettil SV Scientific reports (2018) 81: 17419. . ICC; KD verified; tested species: mouse

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/142104">https://sysy.com/product/142104</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.