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# **Piccolo**

Cat.No. 142 002; Polyclonal rabbit antibody, 200 µl antiserum (lyophilized)

## **Data Sheet**

Reconstitution/ Storage	200 $\mu$ l antiserum, lyophilized. For <b>reconstitution</b> add 200 $\mu$ 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: 5000 (ECL detection) (see remarks) IP: not tested yet ICC: 1: 200 up to 1: 500 IHC: yes IHC_P: not tested yet
Immunogen	Recombinant protein corresponding to AA 4439 to 4776 from rat Piccolo (UniProt Id: Q9JKS6)
Reactivity	Reacts with: rat (Q9JKS6), mouse (Q9QYX7). Other species not tested yet.
Specificity	K.O. PubMed: <u>27537483</u>
Matching control	142-0P
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 142 002

Molecular dynamics of photoreceptor synapse formation in the developing chick retinal

Wahlin KJ, Moreira EF, Huang H, Yu N, Adler R

The Journal of comparative neurology (2008) 5065: 822-37.. WB, IHC

ADAM10 hyperactivation acts on piccolo to deplete synaptic vesicle stores in Huntington's disease.

Cozzolino F, Vezzoli E, Cheroni C, Besusso D, Conforti P, Valenza M, Iacobucci I, Monaco V, Birolini G, Bombaci M, Falqui A, et al. Human molecular genetics (2021):.. WB, IP; tested species: mouse

No symphony without bassoon and piccolo: changes in synaptic active zone proteins in Huntington's disease.

Huang TT, Smith R, Bacos K, Song DY, Faull RM, Waldvogel HJ, Li JY

Acta neuropathologica communications (2020) 81: 77. . ICC, IHC; tested species: human, mouse

The metabolite p-cresol impairs dendritic development, synaptogenesis, and synapse function in hippocampal neurons: Implications for autism spectrum disorder.

Guzmán-Salas S, Weber A, Malci A, Lin X, Herrera-Molina R, Cerpa W, Dorador C, Signorelli J, Zamorano P Journal of neurochemistry (2022) 1614: 335-349. ICC; tested species: rat

Neurexins play a crucial role in cerebellar granule cell survival by organizing autocrine machinery for neurotrophins.

Uemura T, Suzuki-Kouyama E, Kawase S, Kurihara T, Yasumura M, Yoshida T, Fukai S, Yamazaki M, Fei P, Abe M, Watanabe M, et

Cell reports (2022) 391: 110624. . ICC; tested species: mouse

Coordinated bi-directional trafficking of synaptic vesicle and active zone proteins in peripheral nerves.

Juranek JK, Mukherjee K, Jahn R, Li JY

Biochemical and biophysical research communications (2021) 559: 92-98. . IHC; tested species: rat

Neuronal Autophagy Regulates Presynaptic Neurotransmission by Controlling the Axonal Endoplasmic Reticulum. Kuijpers M, Kochlamazashvili G, Stumpf A, Puchkov D, Swaminathan A, Lucht MT, Krause E, Maritzen T, Schmitz D, Haucke V Neuron (2021) 1092: 299-313.e9. . ICC; tested species: mouse

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. . WB; KO verified; tested species: rat

Identification of Potential Interacting Proteins With the Extracellular Loops of the Neuronal Glycoprotein M6a by TMT/MS. Aparicio GI, Formoso K, León A, Frasch AC, Scorticati C

Frontiers in synaptic neuroscience (2020) 12: 28. . ICC; tested species: rat

Interaction of Axonal Chondrolectin with Collagen XIXa1 Is Necessary for Precise Neuromuscular Junction Formation.

Oprisoreanu AM, Smith HL, Arya S, Webster R, Zhong Z, Wehner D, Cardozo MJ, Becker T, Talbot K, Becker CG

Cell reports (2019) 295: 1082-1098.e10. . IHC; tested species: mouse

Visualization of Synchronous or Asynchronous Release of Single Synaptic Vesicle in Active-Zone-Like Membrane Formed on Neuroligin-Coated Glass Surface.

Funahashi J, Tanaka H, Hirano T

Frontiers in cellular neuroscience (2018) 12: 140. . ICC; tested species: rat

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/142002">https://sysy.com/product/142002</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.