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# Ribeye A-domain

Cat.No. 192 103; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

### **Data Sheet**

Reconstitution/ Storage	50 μg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For <b>reconstitution</b> add 50 μl 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	WB: 1: 1000 (AP staining) IP: not tested yet ICC: not tested yet IHC: 1: 200 up to 1: 500 (see remarks) IHC-P: 1: 200
Immunogen	Recombinant protein corresponding to AA 95 to 207 from rat Ribeye (UniProt Id: Q9EQH5-2)
Reactivity	Reacts with: rat (Q9EQH5-2), mouse (P56546-2), human (P56545-2). Other species not tested yet.
Specificity	This antibody recognizes only ribeye and not CtBP 2. K.O. validated PubMed: 35742873
Remarks	<b>IHC</b> : For optimal results, mild fixation (immersion fixation with 4% PFA for 15 min) according to Gierke et al. 2023 is recommended.

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

#### Background

The photoreceptor ribbon synapse is a unique type of synapse specialized for the tonic release of neurotransmitter in the dark. **Ribeye** is a self-aggregating protein and is one of the major scaffolding components of the ribbon on which the neurotransmitter containing vesicles are tethered. The protein consists of a unique A-domain and a B-domain. With the exception of the first 20 amino acids the B-domain is identical to the transcriptional corepressor CtBP 2. Both proteins originate from the same gene.

#### Selected References for 192 103

 $\alpha 2\delta$ -4 is required for the molecular and structural organization of rod and cone photoreceptor synapses.

Kerov V, Laird JG, Joiner ML, Knecht S, Soh D, Hagen J, Gardner SH, Gutierrez W, Yoshimatsu T, Bhattarai S, Puthussery T, et al. The Journal of neuroscience: the official journal of the Society for Neuroscience (2018):.. WB, IHC; tested species: mouse

CaBP1 and 2 enable sustained CaV1.3 calcium currents and synaptic transmission in inner hair cells.

Oestreicher D, Chepurwar S, Kusch K, Rankovic V, Jung S, Strenzke N, Pangrsic T

eLife (2024) 13:.. IHC; tested species: mouse

Two new mouse alleles of Ocm and Slc26a5.

Lachgar-Ruiz M, Ingham NJ, Martelletti E, Chen J, James E, Panganiban C, Lewis MA, Steel KP

Hearing research (2024) 452: 109109. . IHC; tested species: mouse

Presynaptic Nrxn3 is essential for ribbon-synapse maturation in hair cells.

Jukic A, Lei Z, Cebul ER, Pinter K, Tadesse Y, Jarysta A, David S, Mosqueda N, Tarchini B, Kindt K

Development (Cambridge, England) (2024):.. IHC; tested species: mouse

Ectopic Rod Photoreceptor Development in Mice with Genetic Deficiency of WNT2B.

Blomfield AK, Maurya M, Bora K, Pavlovich MC, Yemanyi F, Huang S, Fu Z, O'Connell AE, Chen J

Cells (2023) 127:.. IHC; tested species: mouse

Eliminating Synaptic Ribbons from Rods and Cones Halves the Releasable Vesicle Pool and Slows Down Replenishment.

Mesnard CS, Barta CL, Sladek AL, Zenisek D, Thoreson WB

International journal of molecular sciences (2022) 2312: . . IHC; KO verified; tested species: mouse

RIBEYE B-Domain Is Essential for RIBEYE A-Domain Stability and Assembly of Synaptic Ribbons.

Shankhwar S, Schwarz K, Katiyar R, Jung M, Maxeiner S, Südhof TC, Schmitz F

Frontiers in molecular neuroscience (2022) 15: 838311.. WB; tested species: mouse

Rapid 3D-STORM imaging of diverse molecular targets in tissue.

Albrecht NE, Jiang D, Akhanov V, Hobson R, Speer CM, Robichaux MA, Samuel MA

Cell reports methods (2022) 27: 100253.. IHC; tested species: human, mouse

The SNARE protein SNAP-25 is required for normal exocytosis at auditory hair cell ribbon synapses.

Calvet C, Peineau T, Benamer N, Cornille M, Lelli A, Plion B, Lahlou G, Fanchette J, Nouaille S, Boutet de Monvel J, Estivalet A, et

iScience (2022) 2512: 105628. . IHC: tested species: mouse

Neuroplastin genetically interacts with Cadherin 23 and the encoded isoform Np55 is sufficient for cochlear hair cell function and hearing.

Newton S, Kong F, Carlton AJ, Aguilar C, Parker A, Codner GF, Teboul L, Wells S, Brown SDM, Marcotti W, Bowl MR, et al. PLoS genetics (2022) 181: e1009937... IHC: tested species: mouse

Volumetric super-resolution imaging by serial ultrasectioning and stochastic optical reconstruction microscopy in mouse neural

Vatan T, Minehart JA, Zhang C, Agarwal V, Yang J, Speer CM

STAR protocols (2021) 24: 100971.. IHC; tested species: mouse

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/192103">https://sysy.com/product/192103</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.