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# Rab3a

Cat.No. 107 111; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

# **Data Sheet**

Reconstitution/ Storage	100 $\mu$ g purified IgG, lyophilized. Albumin and azide were added for stabilization. For <b>reconstitution</b> add 100 $\mu$ 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	WB: 1: 1000 (AP staining) IP: yes ICC: 1: 1000 up to 1: 2000 IHC: 1: 500 IHC-P: 1: 2000 up to 1: 3000 DNA-PAINT: external data (see remarks) EM: external data (see remarks) ELISA: yes
Clone	42.2
Subtype	IgG1 (κ light chain)
Immunogen	Full length rat recombinant Rab3a (UniProt Id: P63012)
Epitop	AA 191 to 220 from rat Rab3a (UniProt Id: P63012)
Reactivity	Reacts with: human (P20336), rat (P63012), mouse (P63011), mammals. Other species not tested yet.
Specificity	K.O. validated PubMed: <u>12244319</u>
Remarks	DNA-PAINT: This antibody has been successfully applied and published for this method by customers (see application-specific references).  EM: This antibody has been successfully applied and published for this method by customers (see application-specific references).  ELISA: The ELISA-protocol for membrane proteins is required.  Suitable as capture antibody for sandwich-ELISA. Please refer to the protocol for suitable detector antibodies.

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

### Background

Rab3 proteins belong to the Rab family, a subset of the Ras-related superfamily of small monomeric GTPases. There are four known isoforms: Rab3a, Rab3b, Rab3c, and Rab3d (1, 2). Rab3a and Rab3c are primarily found in neuronal and neuroendocrine cells, whereas Rab3b and Rab3d are predominantly expressed in non-neuronal tissues such as the parotid gland, pancreas, mast cells, and adipose tissue (2, 3).

Rab3a, the most abundant and well-characterized isoform, is highly enriched in synaptic vesicles. It regulates vesicle transport, docking, fusion, and Ca<sup>2</sup>II-dependent neurotransmitter release by cycling between a GDP-bound inactive state and a GTP-bound vesicle-associated active state, interacting with other synaptic proteins in the process (1, 2).

Unlike integral membrane proteins of synaptic vesicles, Rab3a and Rab3c are absent from the Golgi complex, preventing immunostaining of the axo-dendritic region, which can occur with proteins such as synaptophysin, synaptobrevin/VAMP, or synaptogyrin (1).

Beyond the nervous system, Rab3a is also expressed in the pancreas, where it is predominantly localized to  $\beta$ -cells of the islets of Langerhans. It plays a crucial role in regulated insulin secretion, while Rab3d is primarily involved in exocrine pancreatic enzyme secretion (3).

#### Selected References for 107 111

Association of Rab3A with synaptic vesicles at late stages of the secretory pathway.

Matteoli M, Takei K, Cameron R, Hurlbut P, Johnston PA, Südhof TC, Jahn R, De Camilli P
The Journal of cell biology (1991) 1153: 625-33. ICC, IHC, WB, EM; tested species: rat, frog

Quantitative analysis of synaptic vesicle Rabs uncovers distinct yet overlapping roles for Rab3a and Rab27b in Ca2+-triggered exocytosis.

Pavlos NJ, Grønborg M, Riedel D, Chua JJ, Boyken J, Kloepper TH, Urlaub H, Rizzoli SO, Jahn R The Journal of neuroscience: the official journal of the Society for Neuroscience (2010) 3040: 13441-53.. WB, IP, ICC

Actions of Rab27B-GTPase on mammalian central excitatory synaptic transmission.

Arias-Hervert ER, Xu N, Njus M, Murphy GG, Hou Y, Williams JA, Lentz SI, Ernst SA, Stuenkel EL

Physiological reports (2020) 89: e14428. . WB, IHC; tested species: mouse

JIP3 localises to exocytic vesicles and focal adhesions in the growth cones of differentiated PC12 cells. Caswell PT, Dickens M

Molecular and cellular biochemistry (2017):.. WB, ICC; tested species: rat

BDNF enhances spontaneous and activity-dependent neurotransmitter release at excitatory terminals but not at inhibitory terminals in hippocampal neurons.

Shinoda Y, Ahmed S, Ramachandran B, Bharat V, Brockelt D, Altas B, Dean C

Frontiers in synaptic neuroscience (2014) 6: 27. . WB, ICC; tested species: rat

Myosin5a tail associates directly with Rab3A-containing compartments in neurons.

Wöllert T, Patel A, Lee YL, Provance DW, Vought VE, Cosgrove MS, Mercer JA, Langford GM

The Journal of biological chemistry (2011) 28616: 14352-61.. WB, ICC; tested species: mouse

Biochemical, molecular and behavioral phenotypes of Rab3A mutations in the mouse.

Yang S, Farias M, Kapfhamer D, Tobias J, Grant G, Abel T, Bućan M

Genes, brain, and behavior (2007) 61: 77-96. . WB, IP

Rabphilin knock-out mice reveal that rabphilin is not required for rab3 function in regulating neurotransmitter release. Schlüter OM, Schnell E, Verhage M, Tzonopoulos T, Nicoll RA, Janz R, Malenka RC, Geppert M, Südhof TC The Journal of neuroscience: the official journal of the Society for Neuroscience (1999) 1914: 5834-46. . WB, IHC

Synaptic targeting of rabphilin-3A, a synaptic vesicle Ca2+/phospholipid-binding protein, depends on rab3A/3C. Li C, Takei K, Geppert M, Daniell L, Stenius K, Chapman ER, Jahn R, De Camilli P, Südhof TC Neuron (1994) 134: 885-98. WB. IHC

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/107111">https://sysy.com/product/107111</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.