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# Rab5

Cat.No. 108 011; 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 up to 1: 10000 (AP staining) IP: yes ICC: 1: 100 up to 1: 1000 (see remarks) IHC: not tested yet IHC-P: not tested yet
Clone	621.3
Subtype	IgG2a (κ light chain)
Immunogen	Recombinant protein corresponding to AA 1 to 215 from rat Rab5 (UniProt Id: O88565)
Reactivity	Reacts with: human (Q9UJ41), rat (M0RC99), mouse (Q9JM13), hamster. No signal: zebrafish. Other species not tested yet.
Specificity	Specific for rab 5 (probably only rab 5a). No cross-reactivity to other rab proteins.
Remarks	<ul> <li>Recommended for human samples.</li> <li>This antibody was used very successfully for immunoisolation of early endosomes and for the differentiation of early endosomes from related trafficking organelles in neurons and nonneuronal cells.</li> <li>ICC: Not suitable for ICC of PFA-fixed cells. Methanol fixation is recommended.</li> </ul>

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

#### Background

**Rab 5** is a member of the Rab protein family that belongs to the ras-related superfamily of small monomeric GTPases. Rab 5 is ubiquitously expressed in all tissues where it functions in the fusion of early endosomes which is the first fusion step of endocytic organelles after their formation and detachment from the plasma membrane. It is presently the best marker with selectivity for this compartment.

#### Selected References for 108 011

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

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Critical role for piccolo in synaptic vesicle retrieval.

Ackermann F, Schink KO, Bruns C, Izsvák Z, Hamra FK, Rosenmund C, Garner CC

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Neuronal lysosomal dysfunction releases exosomes harboring APP C-terminal fragments and unique lipid signatures. Miranda AM, Lasiecka ZM, Xu Y, Neufeld J, Shahriar S, Simoes S, Chan RB, Oliveira TG, Small SA, Di Paolo G

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Dendritic Cell-Secreted Cytotoxic T-Lymphocyte-Associated Protein-4 Regulates the T-cell Response by Downmodulating Bystander Surface B7.

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The serine/threonine kinase Ndr2 controls integrin trafficking and integrin-dependent neurite growth.

Rehberg K, Kliche S, Madencioglu DA, Thiere M, Müller B, Meineke BM, Freund C, Budinger E, Stork O

The Journal of neuroscience: the official journal of the Society for Neuroscience (2014) 3415: 5342-54. . WB, ICC

Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G

Neuron (2006) 522: 293-305. . ICC, WB

Sunday driver interacts with two distinct classes of axonal organelles.

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Sugar transporter Slc37a2 regulates bone metabolism in mice via a tubular lysosomal network in osteoclasts.

Ng PY, Ribet ABP, Guo Q, Mullin BH, Tan JWY, Landao-Bassonga E, Stephens S, Chen K, Yuan J, Abudulai L, Bollen M, et al.

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Depletion of the AD Risk Gene SORL1 Selectively Impairs Neuronal Endosomal Traffic Independent of Amyloidogenic APP Processing.

Knupp A, Mishra S, Martinez R, Braggin JE, Szabo M, Kinoshita C, Hailey DW, Small SA, Jayadev S, Young JE Cell reports (2020) 319: 107719. . ICC; tested species: human

CtBP1-Mediated Membrane Fission Contributes to Effective Recycling of Synaptic Vesicles.

Ivanova D, Imig C, Camacho M, Reinhold A, Guhathakurta D, Montenegro-Venegas C, Cousin MA, Gundelfinger ED, Rosenmund C, Cooper B, Fejtova A, et al.

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MHC class I and II peptide homology regulates the cellular immune response.

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Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/108011">https://sysy.com/product/108011</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.