SYSY Antibodies

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EEA1

Cat.No. 237 002; Polyclonal rabbit antibody, 200 µl antiserum (lyophilized)

Data Sheet

Reconstitution/ Storage	200 µl antiserum, lyophilized. For **reconstitution** add 200 µl H_2O, 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 (AP staining) **IP**: not tested yet **ICC**: 1 : 1000 up to 1 : 2000 **IHC**: not recommended **IHC-P (FFPE)**: 1 : 200
Immunogen	Synthetic peptide corresponding to AA 2 to 13 from human EEA1 (UniProt Id: Q15075)
Reactivity	Reacts with: human (Q15075), rat (A0A0G2K051), mouse (Q8BL66). No signal: zebrafish. Other species not tested yet.

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

Background

Extracellular compounds are internalized by endocytosis into so called endocytic vesicles. They fuse with early endosomes, from where the endocytosed material can be shuttled to a number of alternative destinations.
Early **e**ndosomal **a**ntigen **1** (**EEA 1**) is a peripheral membrane protein that locates to early endosomes via binding to the membrane lipid phosphatidylinositol 3-phosphate (PtdIns3P) and the active form of Rab5.
Autoantibodies against EEA 1 have been shown to be associated with subacute cutaneous systemic lupus erythematosus.

Selected References for 237 002

A novel method for culturing stellate astrocytes reveals spatially distinct Ca2+ signaling and vesicle recycling in astrocytic processes.
Wolfes AC, Ahmed S, Awasthi A, Stahlberg MA, Rajput A, Magruder DS, Bonn S, Dean C
The Journal of general physiology (2017) 1491: 149-170. . **ICC**

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**

C9orf72 hexanucleotide repeat expansions impair microglial response in ALS.
Masrori P, Bijnens B, Fumagalli L, Davie K, Poovathingal SK, Meese T, Storm A, Hersmus N, Fazal R, van den Biggelaar D, Asselbergh B, et al.
Nature neuroscience (2025) : . . **ICC; tested species: human**

Coupling of microtubule motors with AP-3 generated organelles in axons by NEEP21 Family Member Calcyon.
Shi L, Hines T, Bergson C, Smith D
Molecular biology of the cell (2018) : mbcE18010007. . **ICC; tested species: monkey**

Molecular anatomy of a trafficking organelle.
Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, et al.
Cell (2006) 1274: 831-46. . **WB**

Sorting in early endosomes reveals connections to docking- and fusion-associated factors.
Barysch SV, Aggarwal S, Jahn R, Rizzoli SO
Proceedings of the National Academy of Sciences of the United States of America (2009) 10624: 9697-702. .

SNARE function is not involved in early endosome docking.
Geumann U, Barysch SV, Hoopmann P, Jahn R, Rizzoli SO
Molecular biology of the cell (2008) 1912: 5327-37. .

Selected General References

Cell-cycle-dependent binding kinetics for the early endosomal tethering factor EEA1.
Bergeland T et al. EMBO Rep. (2008) PubMed:18188183

EEA1, a tethering protein of the early sorting endosome, shows a polarized distribution in hippocampal neurons, epithelial cells, and fibroblasts.
Wilson JM et al. Mol. Biol. Cell (2000) PubMed:10930461

The Rab5 effector EEA1 interacts directly with syntaxin-6.
Simonsen A et al. J. Biol. Chem. (1999) PubMed:10506127

The endosome fusion regulator early-endosomal autoantigen 1 (EEA1) is a dimer.
Callaghan J et al. Biochem. J. (1999) PubMed:10024533

Access the online factsheet including applicable protocols at https://sysy.com/product/237002 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable 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. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.