SYSY Antibodies

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Uncoating ATPase (HSC70)

Cat.No. 149 011; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized. Albumin and azide were added for stabilization. For **reconstitution** add 100 µl H_2O 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 : 500 up to 1 : 5000 (AP staining) **IP**: not tested yet **ICC**: 1 : 500 **IHC**: external data (see remarks) **IHC-P (FFPE)**: 1 : 200
Clone	3C5
Subtype	IgG1 (κ light chain)
Immunogen	Recombinant protein corresponding to AA 1 to 650 from bovine Uncoating ATPase (UniProt Id: P19120)
Epitop	AA 391 to 546 from bovine Uncoating ATPase (UniProt Id: P19120)
Reactivity	Reacts with: human (P11142), rat (P63018), mouse (P63017), hamster, cow. Other species not tested yet.
Remarks	When microinjected into cells, the antibody inhibits clathrin mediated endocytosis. **IHC**: This antibody has been successfully applied and published for this method by customers (see application-specific references). It has not been validated using our standard protocols.

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

Background

Clathrin coated vesicles are involved in a wide variety of transport events of the eucaryotic cell. This includes receptor mediated endocytosis or the recycling of synaptic vesicles in nerve terminals. Before the vesicles can fuse with the endosomal compartment the clathrin coat has to be removed in an ATP dependent process. **Uncoating ATPase** (UA), also referred to as **hsc70**, has been shown to be functionally involved in this process.
Uncoating ATPase belongs to the heatshock protein (hsp70) family. Most proteins of this family are molecular chaperones and the uncoating ATPase shares the ability to refold proteins in several cellular processes.

Selected References for 149 011

Chaperone-mediated autophagy in neuronal dendrites utilizes activity-dependent lysosomal exocytosis for protein disposal.
Grochowska KM, Sperveslage M, Raman R, Failla AV, Głów D, Schulze C, Laprell L, Fehse B, Kreutz MR
Cell reports (2023) 428: 112998. . **ICC; tested species: rat**

Synaptic AP2 CCV life cycle regulation by the Eps15, ITSN1, Sgip1/AP2, synaptojanin1 interactome.
Mishra R, Sengül GF, Candiello E, Schu P
Scientific reports (2021) 111: 8007. . **IP; tested species: mouse**

Evidence for a Clathrin-independent mode of endocytosis at a continuously active sensory synapse.
Fuchs M, Brandstätter JH, Regus-Leidig H
Frontiers in cellular neuroscience (2014) 8: 60. . **IHC; tested species: rat**

Cysteine string protein-alpha prevents activity-dependent degeneration in GABAergic synapses.
García-Junco-Clemente P, Cantero G, Gómez-Sánchez L, Linares-Clemente P, Martínez-López JA, Luján R, Fernández-Chacón R
The Journal of neuroscience : the official journal of the Society for Neuroscience (2010) 3021: 7377-91. . **WB**

Proteomic analysis reveals the composition of glutamatergic organelles of auditory inner hair cell.
Cepeda AP, Ninov M, Neef J, Parfentev I, Kusch K, Reisinger E, Jahn R, Moser T, Urlaub H
Molecular & cellular proteomics : MCP (2023) : 100704. . **IHC; tested species: mouse**

Hsc70 phosphorylation patterns and calmodulin regulate AP2 CCV life span for cell adhesion protein transport.
Sengül GF, Mishra R, Candiello E, Schu P
Biochimica et biophysica acta. Molecular cell research (2023) : 119611. . **WB; tested species: mouse**

CSPα reduces aggregates and rescues striatal dopamine release in α-synuclein transgenic mice.
Caló L, Hidari E, Wegrzynowicz M, Dalley JW, Schneider BL, Podgajna M, Anichtchik O, Carlson E, Klenerman D, Spillantini MG
Brain : a journal of neurology (2021) 1446: 1661-1669. . **WB; tested species: mouse**

Aggregation of mutant cysteine string protein-α via Fe-S cluster binding is mitigated by iron chelators.
Naseri NN, Ergel B, Kharel P, Na Y, Huang Q, Huang R, Dolzhanskaya N, Burré J, Velinov MT, Sharma M
Nature structural & molecular biology (2020) 272: 192-201. . **WB; tested species: mouse**

Lysosomal dysfunction disrupts presynaptic maintenance and restoration of presynaptic function prevents neurodegeneration in lysosomal storage diseases.
Sambri I, D'Alessio R, Ezhova Y, Giuliano T, Sorrentino NC, Cacace V, De Risi M, Cataldi M, Annunziato L, De Leonibus E, Fraldi A, et al.
EMBO molecular medicine (2017) 91: 112-132. . **WB; tested species: mouse**

A mouse model for fucosidosis recapitulates storage pathology and neurological features of the milder form of the human disease.
Wolf H, Damme M, Stroobants S, D'Hooge R, Beck HC, Hermans-Borgmeyer I, Lüllmann-Rauch R, Dierks T, Lübke T
Disease models & mechanisms (2016) 99: 1015-28. . **WB; tested species: mouse**

The SNARE protein vti1a functions in dense-core vesicle biogenesis.
Walter AM, Kurps J, de Wit H, Schöning S, Toft-Bertelsen TL, Lauks J, Ziomkiewicz I, Weiss AN, Schulz A, Fischer von Mollard G, Verhage M, et al.
The EMBO journal (2014) 3315: 1681-97. . **WB; tested species: mouse**

Access the online factsheet including applicable protocols at https://sysy.com/product/149011 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.