

Uncoating ATPase

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 ₂ 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 : 500 up to 1 : 5000 (AP staining) IP: not tested yet ICC: 1 : 500 IHC: yes IHC_P: 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	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	For fixation a 20 min treatment with 3.7 % PFA in PBS is recommended. When microinjected into cells, the antibody inhibits clathrin mediated endocytosis.

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, synaptotagmin1 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 our antibodies and control proteins / peptides are shipped lyophilized (vacuum freeze-dried) 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 freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.