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# **Amphiphysin**

Cat.No. 120 003; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

## **Data Sheet**

Reconstitution/ Storage	50 $\mu g$ specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For <b>reconstitution</b> add 50 $\mu$ 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: not tested yet ICC: 1: 500 IHC: not tested yet IHC-P: not tested yet
Immunogen	Synthetic peptide corresponding to AA 2 to 15 from rat Amphiphysin (UniProt Id: 008838)
Reactivity	Reacts with: human (P49418), rat (O08838), mouse (Q7TQF7), hamster, cow, chicken. Other species not tested yet.
Specificity	K.D. validated PubMed: 37243578
Matching control	120-0P

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

# Background

**Amphiphysin**, a dominant autoantigen in paraneoplastic Stiff-man syndrome, is a neuronal protein highly concentrated in nerve terminals. It is an abundant presynaptic protein that interacts via its COOH-terminal src homology 3 (SH3) domain with the GTPase dynamin and the inositol-5-phosphatase synaptojanin and additionally directly to clathrin.

Amphiphysin, dynamin and synaptojanin have a putative role in synaptic vesicle recycling and undergo rapid dephosphorylation in synaptosomes stimulated by a depolarizing stimulus. Two isoforms are known which seem to act in concert as a heterodimer.

### Selected References for 120 003

Spatial proteomics in neurons at single-protein resolution.

Unterauer EM, Shetab Boushehri S, Jevdokimenko K, Masullo LA, Ganji M, Sograte-Idrissi S, Kowalewski R, Strauss S, Reinhardt SCM, Perovic A, Marr C, et al.

Cell (2024) 1877: 1785-1800.e16. . DNA PAINT; tested species: rat

 $The \ phospho-regulated \ amphiphysin/endophilin\ interaction\ is\ required\ for\ synaptic\ vesicle\ endocytosis.$ 

Kontaxi C, Kim N, Cousin MA

Journal of neurochemistry (2023) 1662: 248-264. . ICC; KD verified; tested species: mouse

### **Selected General References**

Accessory factors in clathrin-dependent synaptic vesicle endocytosis. Slepnev VI et al. Nat. Rev. Neurosci. (2000) PubMed:11257904

The structural era of endocytosis.

Marsh M et al. Science (1999) PubMed:10398591

The amphiphysin family of proteins and their role in endocytosis at the synapse.

Wigge P et al. Trends Neurosci. (1998) PubMed:9720601

Amphiphysin heterodimers: potential role in clathrin-mediated endocytosis.

Wigge P et al. Mol. Biol. Cell (1997) PubMed:9348539

 $A\ role\ of\ amphiphysin\ in\ synaptic\ vesicle\ endocytosis\ suggested\ by\ its\ binding\ to\ dynamin\ in\ nerve\ terminals.$ 

David C et al. Proc. Natl. Acad. Sci. U.S.A. (1996) PubMed:8552632

The synaptic vesicle cycle: a cascade of protein-protein interactions.

Südhof TC et al. Nature (1995) PubMed:7791897

Synaptic vesicles and exocytosis.

Jahn R et al. Annu. Rev. Neurosci. (1994) PubMed:8210174

The synaptic vesicle-associated protein amphiphysin is the 128-kD autoantigen of Stiff-Man syndrome with breast cancer. De Camilli P et al. J. Exp. Med. (1993) PubMed:8245793

Amphiphysin, a novel protein associated with synaptic vesicles.

Lichte B et al. EMBO J. (1992) PubMed:1628617

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/120003">https://sysy.com/product/120003</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.