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# ATP1A2 Cytoplasmic loop

Cat.No. 512 015; Polyclonal Guinea pig antibody, 50 µg specific antibody (lyophilized)

## Data Sheet

Reconstitution/ Storage	50 μg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For <b>reconstitution</b> add 50 μl 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 : 2000 (AP-staining) ICC: 1 : 500 up to 1 : 1000 IHC: 1 : 500 IHC-P: 1 : 500
Immunogen	Synthetic peptide corresponding to residues surrounding AA 550 from human ATP1A2 (UniProt Id: P50993)
Reactivity	Reacts with: rat (P06686), mouse (Q6PIE5). Other species not tested yet.
Specificity	The peptide sequence used for immunization is specific for subunit alpha-2 and should not recognize the other subunits alpha 1, 3 or 4.

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

Background

**ATP1A2**, also referred to as **Sodium/potassium-transporting ATPase subunit alpha-2**, is the catalytic subunit of the enzyme, which catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the plasma membrane. This process is essential for maintaining the asymmetrical distribution of Na<sup>+</sup> and K<sup>+</sup> ions and the resting membrane potential, thereby providing the energy needed for active nutrient transport.

A functional Na<sup>+</sup>/K<sup>+</sup> ATPase consists of a catalytic  $\alpha$ -subunit, which contains the ATP-binding sites, and a smaller  $\beta$ -subunit, necessary for full enzymatic activity and serving as an anchoring protein. Auxiliary proteins, such as  $\gamma$  subunits (FXYD polypeptides), further modify the enzyme's transport properties. Currently, four catalytic ( $\alpha$ ) and two glycoprotein ( $\beta$ ) subunits have been identified, each distributed in a highly tissue- and cell-specific manner. In the brain, three  $\alpha$  subunit isoforms ( $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 3) are expressed: ATP1A1 is present in all cell types, ATP1A3 is primarily found in neurons, and **ATP1A2** is almost exclusively expressed in glial cells (1).

Specific roles for glial Na<sup>+</sup>/K<sup>+</sup>-ATPases, and specifically the  $\alpha$ 2-subunit containing enzyme include a contribution to K<sup>+</sup> homeostasis and to neurotransmitter (notably glutamate) clearing from the synaptic cleft. Interestingly, it has been shown that a glial Sodium/potassium-transporting ATPase containing the  $\alpha$ 2 subunit is recruited in response to glutamate uptake and is necessary to couple neuronal activity to glucose utilization (2).

## **Selected General References**

Region- and neuronal-subtype-specific expression of Na,K-ATPase alpha and beta subunit isoforms in the mouse brain. Murata K et al. J Comp Neurol (2020) PubMed:32301109

Similar perisynaptic glial localization for the Na+,K+-ATPase alpha 2 subunit and the glutamate transporters GLAST and GLT-1 in the rat somatosensory cortex.

Cholet N et al. Cereb Cortex (2002) PubMed:11950769

Glutamate uptake stimulates Na+,K+-ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. Pellerin L et al. J Neurochem (1997) PubMed:9349559

Access the online factsheet including applicable protocols at <u>https://sysy.com/product/512015</u> 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.