SYSY Antibodies

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α-Tubulin

Cat.No. 302 209; Recombinant chicken antibody, 50 µg recombinant IgY (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg purified recombinant IgY, lyophilized. Albumin and azide were added for stabilization. For **reconstitution** add 50 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 : 1000 up to 1 : 10000 (AP-staining) **ICC**: 1 : 1000 up to 1 : 2000 (see remarks) **IHC**: 1 : 500 up to 1 : 1000 **IHC-P**: 1 : 500 up to 1 : 1000
Clone	Ch3A2
Subtype	IgY (λ light chain)
Immunogen	Synthetic peptide corresponding to AA 419 to 435 from human α-tubulin 4A (UniProt Id: P68366)
Reactivity	Reacts with: human (P68366), rat (Q5XIF6), mouse (P68368), vertebrates, invertebrates, yeast. Other species not tested yet.
Specificity	Specific for α-tubulin (glu- and tyr-α-tubulin)
Matching control	302-21P
Remarks	This antibody is a chimeric antibody based on the well known monoclonal mouse antibody clone 3A2. The constant regions of the heavy and light chains have been replaced with chicken specific sequences. Therefore, the antibody can be used with standard anti-chicken secondary reagents. The antibody has been expressed in mammalian cells. **ICC**: Methanol or methanol-acetone fixation is possible.

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

Background

Microtubules are involved in a wide variety of cellular activities ranging from mitosis and transport events to cell movement and the maintainance of cell shape.
Tubulin itself is a globular protein which consists of two polypeptides, **α-tubulin** and β-tubulin. α- and β-tubulin dimers are assembled to 13 protofilaments that form a microtubule of 22 nm diameter. Assembled microtubules can be detyrosinated by a carboxypeptidaseS called vasohibins / SVBPs. Detyrosinated α-tubulin is referred to as **Glu-α-tubulin** and occurs for exemple in neurons. This reaction can be reverted by Tubulin tyrosine ligase (TTL) that ads a C-terminal tyrosin to Glu α-tubulin. Another post-translational modification of α-tubulin is C-terminal polyglutamylation which is also characteristic for microtubules in neuronal cells and the mitotic spindle. A third variant of detyrosinated α-tubulin is **Δ2-tubulin** which lacks the C-terminal glutamic acid. It cannot be tyrosinated by TTL and is one of the dominant α-tubulin isoforms in neurons.

Selected General References

A vital role of tubulin-tyrosine-ligase for neuronal organization.
Erck C et al. Proc. Natl. Acad. Sci. U.S.A. (2005) PubMed:15899979

Association of tubulin carboxypeptidase with microtubules in living cells.
Contin MA et al. Biochem. J. (1999) PubMed:10191280

Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated, in neuronal tissues and in stable microtubule assemblies.
Paturle-Lafanechère L et al. J. Cell. Sci. (1994) PubMed:7962195

Characterization of the tubulin-tyrosine ligase.
Ersfeld K et al. J. Cell Biol. (1993) PubMed:8093886

Class II tubulin, the major brain beta tubulin isotype is polyglutamylated on glutamic acid residue 435.
Rüdiger M et al. FEBS Lett. (1992) PubMed:1379548

Autoregulation of tubulin synthesis in hepatocytes and fibroblasts.
Caron JM et al. J. Cell Biol. (1985) PubMed:3902854

Autoregulation of tubulin synthesis in enucleated cells.
Caron JM et al. Nature () PubMed:4058574

Access the online factsheet including applicable protocols at https://sysy.com/product/302209 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.