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a-Tubulin

Cat.No. 302 008; Recombinant rabbit antibody, 100 µg recombinant IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 μ g purified recombinant IgG, lyophilized. For reconstitution add 100 μ l 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: 1000 up to 1: 5000 (AP staining) IP: not tested yet ICC: 1: 500 (see remarks) IHC: 1: 500 IHC_P: not tested yet
Clone	RbF2C
Subtype	IgG1 (κ light chain)
Immunogen	α-Tubulin purified from bovine brain
Reactivity	Reacts with: human, rat, mouse, cow. Other species not tested yet.
Specificity	Specific for α-tubulin
Remarks	This antibody is a chimeric antibody based on the monoclonal mouse antibody F2C. The constant regions of the heavy and light chains have been replaced with rabbit specific sequences. The antibody can therefore be used with standard anti-rabbit secondary reagents. The antibody has been expressed in mammalian cells and carries a Strep-tag® at the C-terminus of the heavy chain. ICC: Methanol or PFA fixation

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, \mathbf{a} -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 \mathbf{Glu} - \mathbf{a} -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 Δ 2-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 References for 302 008

SNAP23 depletion enables more SNAP25/calcium channel excitosome formation to increase insulin exocytosis in type 2 diabetes.

Liang T, Qin T, Kang F, Kang Y, Xie L, Zhu D, Dolai S, Greitzer-Antes D, Baker RK, Feng D, Tuduri E, et al. JCI insight (2020) 53:.. WB; tested species: human,mouse

Selected General References

A vital role of tubulin-tyrosine-ligase for neuronal organization.

Erck C, Peris L, Andrieux A, Meissirel C, Gruber AD, Vernet M, Schweitzer A, Saoudi Y, Pointu H, Bosc C, Salin PA, et al. Proceedings of the National Academy of Sciences of the United States of America (2005) 10222: 7853-8.

Association of tubulin carboxypeptidase with microtubules in living cells.

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The Biochemical journal (1999) 339 (Pt 2): 463-71...

Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated, in neuronal tissues and in stable microtubule assemblies.

Paturle-Lafanechère L, Manier M, Trigault N, Pirollet F, Mazarquil H, Job D

Journal of cell science (1994) 107 (Pt 6): 1529-43. .

Characterization of the tubulin-tyrosine ligase.

Ersfeld K. Wehland J. Plessmann U. Dodemont H. Gerke V. Weber K

The Journal of cell biology (1993) 1203: 725-32...

Class II tubulin, the major brain beta tubulin isotype is polyglutamylated on glutamic acid residue 435.

Rüdiger M, Plessman U, Klöppel KD, Wehland J, Weber K

FEBS letters (1992) 3081: 101-5...

Autoregulation of tubulin synthesis in hepatocytes and fibroblasts.

Caron JM, Jones AL, Kirschner MW

The Journal of cell biology (1985) 1015 Pt 1: 1763-72...

Autoregulation of tubulin synthesis in enucleated cells.

Caron JM. Jones AL. Rall LB. Kirschner MW

Nature () 3176038: 648-51. .

Access the online factsheet including applicable protocols at https://sysy.com/product/302008 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.