V. 3.3

Acetylated Tubulin protein
Source: Porcine Brain
Cat. # CS-TAC01
Lot # 013 1 x 500 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called alpha and beta tubulin. The two proteins are encoded by separate genes, or small gene families, whose sequences are highly conserved throughout the eukaryotic kingdom. Post-translational modifications (PTMs) of Microtubules (MTs)

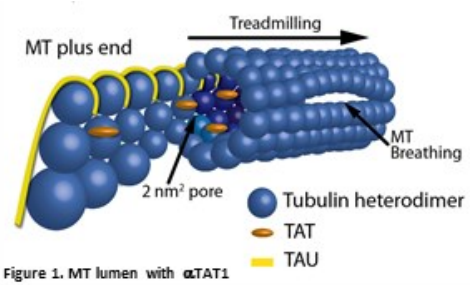

Figure 1. MT lumen with αTAT1

were first discovered over 40 years ago (1) and are a mechanism to regulate MT activity. The acetylation of Lysine 40 (K40) of α-tubulin by the α-tubulin N-acetyltransferase 1 (αTAT1) (Fig. 1) is the most studied acetylated mark of tubulin. Most PTMs of MTs are found on the C-terminus, but K40 is located on the luminal side of MTs. It is unclear whether acetylation of K40 influences MT stability, but recent work by Cryo-EM has observed conformational changes to MTs upon acetylation at K40 (2). In addition, it is believed that PTMs are associated with differential binding of MT-associated proteins (MAPs) and kinesin motor proteins to MTs (3,4). While further studies are needed to better understand the role in which MT acetylation plays in regulating cellular processes, abnormal acetylation has been linked to neurodegenerative disorders, ciliopathies, and cancers (5).

Material

The acetylated tubulin protein (Cat. # CS-TAC01) was prepared from porcine brain by adaptation of the method Shelanski et al (6). Further purification was achieved by cation exchange chromatography, and acetylation of K40 of α-tubulin was facilitated by the αTAT1 protein as reported in Friedmann et al and Howes et al(7, 8). Tubulin consists of a heterodimer of one alpha and one beta isotype, each tubulin isotype is 55 kDa in size (see Figure 2). Typically, the molar equivalent of tubulin is defined as the heterodimer which has a molecular weight of 110 kDa. The protein is supplied as a white solid.

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is desiccated at 4°C. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C.

Reconstitute to 10 mg/ml with General Tubulin Buffer (80 mM PIPES pH 6.9, 2 mM MgCl2, 0.5 mM EGTA.) supplemented with 1 mM GTP. Snap freeze "experiment sized" aliquots in liquid nitrogen and store at -70°C. Reconstituted CS-TAC01 is stable for 6 months at -70°C. Reconstituted CS-TAC01 MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this will result in significant loss of activity.

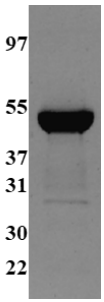

Figure 2. Purity analysis of Acetylated Tubulin Protein Legend: A 10 µg sample of acetylated tubulin protein (Cat. # CS-TAC01) purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. Acetylated tubulin protein was determined to be 90% pure. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark 12).

Biological Activity

Tubulin Polymerization Assay and making a stock of K40 acetylated microtubules.

The biological activity of acetylated tubulin is assessed by a tubulin polymerization assay (Fig. 3). The results indicate K40-Acetylated tubulin shows slightly slower kinetics than native tubulin but it reaches a similar equilibrium point after 900s.

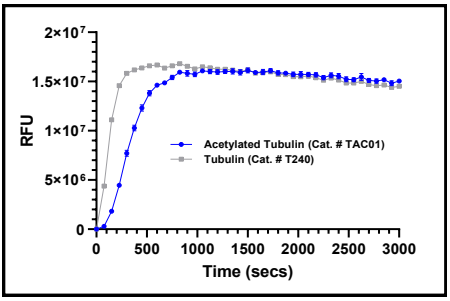

Figure 3. Acetylated Tubulin Polymerization Assay

Figure legend: Each 50 µl well reaction contains 2.5 mg/ml Acety-

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lated tubulin (Cat. # CS-TAC01) (n=2) or 2.5 mg/ml tubulin (Cat. # T240) (n=2). Excitation was at 360 nm and emission at 420 nm measured on an iD5 multi-mode microplate reader (Molecular devices) over 50 minutes at 37°C. The protocol for creating this polymerization assay can be found online at www.cytoskeleton.com/CS-TAC01.

Preparing microtubules for binding studies or cryo-EM

Assuming the 500 µg vial of tubulin was resuspended in 50 µl of General Tubulin Buffer plus 1 mM GTP before freezing in aliquots of 5 µl, as described above, Then follow the protocol:

1. Make a fresh stock of modified G-PEM plus 20% glycerol, using 647 µl General Tubulin Buffer (Cat. # BST01), plus 333 µl of Tubulin glycerol buffer (Cat. # BST05), plus 20 µl of 100 mM GTP solution (Cat. # BST06), place on ice.
2. Take one 5 µl aliquot of frozen tubulin and rapidly defrost in room temperature waterbath.
3. Add 5 µl of G-PEM plus 20% glycerol and mix by pipetting up and down twice.
4. Place at 37°C for 40 min.

These microtubules can be further stabilized with 10 µM paclitaxel (Cat. # TXD01) to make a room temperature stable MT stock.
The final formula for modified G-PEM plus 20% glycerol is 80 mM PIPES pH 6.9, 2.0 mM MgCl2, 0.5 mM EGTA, 2.0 mM GTP and 20% (v/v) glycerol.

Acetylated Tubulin IP with Acetyl-Lysine Signal-Seeker™

The levels of acetylation to Lys40 of α-tubulin (Cat. # CS-TAC01) was compared to that of unmodified tubulin (Cat. # T240).

Reagents

1. Acetylated Tubulin (Cat. # CS-TAC01)
2. Tubulin protein (Cat. # T240)
3. Anti-acetylated tubulin mouse monoclonal antibody (clone 6 -11B-1 Sigma Cat. # T7451) or a pan acetyl-lysine mouse monoclonal antibody from Cytoskeleton (Cat. # AAC02, AAC03, or AAC03-HRP)
4. Acetyl-Lysine Signal-Seeker™ Kit (Cat. # BK163)
5. 1 mg/ml BSA in TBST
6. 100 mM GTP (Cat. # BST06)
7. Modified TBST with 1 mM MgCl2 and 0.5 mM GTP.

Equipment

Western blot equipment and reagents necessary for transferring and developing blots. A centrifuge capable of spinning 1.5 ml tubes at 4°C.

Method (Acetylated tubulin IP assay)

1. Prepare reagent solutions, acetyl lysine affinity beads, and control beads as described in BK163 manual before proceeding.
2. Resuspend tubulin (Cat. # T240) and acetylated tubulin (Cat. # CS-TAC01) in ice cold G-PEM to 0.1 mg/ml working stock solutions.
3. Resuspend Acetyl-lysine (Cat. # AAC04-beads) and ClG02 (Cat. # ClG02) beads with 500 µl each of ice cold Modified TBST buffer.
4. Aliquot 100 µl of BSA solution into three labeled (A,B,C) 1.5 ml tubes on ice.
5. Add 990 µl of Modified TBST to all three tubes.
6. Add 10 µl 0.1 mg/ml (1 µg protein) of acetylated tubulin to tubes A and C, and 10 µl of native tubulin (1 µg protein, Cat. # T240) to tube B.
7. Mix beads slurries just prior to pipetting, adding 50 µl of AAC04 beads to Tubes B and C, and 50 µl of Control beads to tube A.
8. Incubate the tubes on a rotating platform at 4°C for 2h.
9. Collect beads by centrifugation at 5,000 x g for 1 minute at 4°C.
10. Supernatant can be aspirated or saved for unbound analysis (Note: 5% w/v TCA is good for precipitation, and then centrifuge 14k xg to collect pellet and immediately resuspend in 50 µl of 2x non-reducing SDS-PAGE loading buffer).
11. Wash beads in 1 ml Modified TBST.
12. Collect beads by centrifugation at 5,000 x g for 1 minute at 4°C.
13. Aspirate off as much supernatant as possible without disturbing the beads.
14. Repeat the wash step (11-13) once more.
15. After the final wash, completely remove buffer supernatant.
16. Add 50 µl of 2x non-reducing SDS-PAGE loading buffer.
17. Place samples in a boiling water bath for 2 minutes. Collect sample by centrifugation at 10,000 x g for 1 minute at RT.
18. If necessary, freeze samples and stop here, or proceed to running SDS-PAGE and western blot analysis.

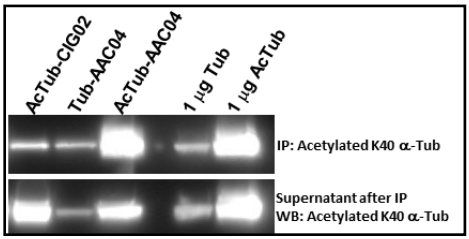

Figure 4. Acetylated Tubulin Immunoprecipitation Assay

Figure legend: IP was performed by incubating 1 µg of acetylated tubulin (AcTub) or 1 µg unmodified tubulin (Tub) with AAC04 or ClG02 control beads. Unbound acetylated tubulin from the supernatant (Step 11) was precipitated out of solution with 5% TCA and run on a separate SDS-PAGE gel. Both blots were probed with 1:30:000 Anti-acetylated K40 α-tubulin mouse monoclonal antibody (clone 6-11B-1 Sigma Cat. # T7451).

References

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