V. 1.0

Alpha Tubulin N-Acetyltransferase 1 (2-236) protein (Human recombinant)
Cat. # CS-TAT01
Lot # 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

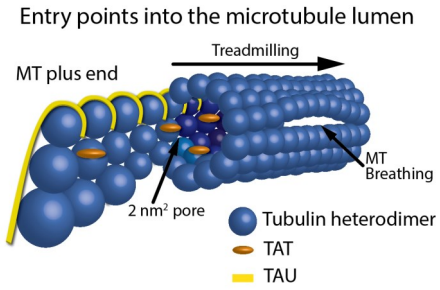

Figure 1. MT lumen with αTAT1

Post-translational modifications (PTMs) of microtubules (MTs) 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 the acetylation of K40 influences MT stability, but recent work using Cryo-EM has observed conformational changes to MTs upon acetylation at K40 (2). In addition, it is believed that MT-PTMs are associated with the differential binding of MT-associated proteins (MAPs) and kinesin motor proteins to MTs (3,4). While further studies are needed to define the role MT acetylation plays in regulating cellular processes, abnormal acetylation has been linked to neurodegenerative disorders, ciliopathies, and cancers (5).

Material

The human αTAT1 protein (2-236) was produced in a bacterial expression system. The recombinant protein is untagged and has an approximate molecular weight of 27 kDa. The protein is lyophilized and supplied as a white solid.

Storage and Reconstitution

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

Reconstitute to 5 mg/ml by the addition of 20 µl of distilled water. The protein will be in the following buffer; 20 mM Tris pH 8.0, 10 mM NaCl, 6% sucrose, and 1% Dextran. Snap freeze "experiment -sized" aliquots in liquid nitrogen and store them at -70°C. Reconstituted αTAT1 is stable for six months at -70°C.

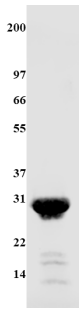

Figure 2. Purity analysis of αTAT1 residues 2-236
Legend: A 20 µg sample of αTAT1 purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. Acetylated tubulin protein was determined to be 90% pure. (see Figure 2). Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark 12).

Biological Activity

α-tubulin K40 acetylation by αTAT1.

The biological activity of αTAT1 is assessed by its ability to acetylate tubulin during tubulin polymerization by measuring tubulin acetylation via Western analysis (Fig. 3). The results show an increase in K40-Acetylated α–tubulin with the addition of αTAT1.

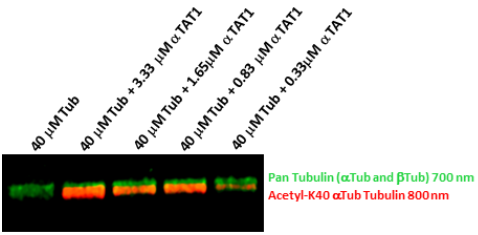

Figure 3. Tubulin acetylation by αTAT1 Figure Legend: Each reaction contains 40 µM of tubulin (Cat. # T240) and decreasing reaction ratios of 1:12, 1:24, 1:48, and 1:120 Tubulin:αTAT1 proteins. Tubulin was polymerized at 5 mg/ml with or without αTAT1 for 60 minutes at 30°C. K40 acetylation of α-tubulin was detected by multiplex Western blotting with a mouse monoclonal antibody (6-11b-1, Sigma) and compared to both α- and β-bands of tubulin with a pan-tubulin antibody (Cat. # ATN02). Secondary antibodies were labeled with near-infrared fluorescent dyes to simultaneously detect total tubulin in the 700 nm channel (green) and acetylated K40 α-tubulin in the 800 nm channel (red).

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α-tubulin K40 acetylation by αTAT1.

Reagents

1. Tubulin protein (Cat. # T240-A)
2. αTAT1 2-236 (Cat. # CS-TAT01)
3. Acetyl coenzyme A Trisodium Salt (Sigma Cat. # A2056)
4. 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)
5. 100 mM GTP (Cat. # BST06)
6. 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 10 µl of 100 mM GTP solution (Cat. # BST06), and 1 µl of DTT, place on ice.

Equipment

Western blot equipment and reagents are necessary for transferring and developing blots.

Method (Acetylated tubulin assay)

1. Resuspend tubulin (Cat. # T240) in ice-cold G-PEM to 5.0 mg/ml working stock solutions.
2. Resuspend αTAT1 (Cat. # CS-TAT01) to 1.0 mg/ml with 100 µl each of ice-cold G-PEM.
3. Make a working stock of 10 mM acetyl coenzyme A (Acetyl-CoA) with cold MilliQ-H_2O.
4. Add the following reagents as directed in Table 1. Keep reactions on ice.

Reaction (T240 : aTAT1 ratio)	T240 5 mg/ml (µl)	10 mM Acetyl-CoA (µl)	αTAT1 1 mg/ml	G-PEM (µl)
1:0	50	1	0	5
1:12	50	1	5	0
1:24	50	1	2.5	2.5
1:48	50	1	1.25	3.25
1:120	50	1	0.5	4.5

5. Mix reactions and spin down. Place at 37° for 5 mins.
6. Transfer reactions to 30° for 60 mins.
7. Spin down reactions and stop by adding 2 µl of each reaction to 598 µl of 2x reducing SDS-PAGE loading buffer.
8. Place samples in a boiling water bath for 2 minutes. Spin down the samples by centrifugation at 10,000 x g for 1 minute at RT.
9. If necessary, freeze samples and stop here, or proceed to run SDS-PAGE and western blot analysis.
10. Load 15 µl of each reaction to SDS-PAGE gel and transfer to immobilion-PVDF (LiCOR Cat. # 928-40004).
11. Transfer blot to a GO-Blot Western blotting device and run pre-defined protocol 2. Probe with 1:40:000 Anti-acetylated K40 α-tubulin mouse monoclonal antibody (clone 6-11B-1 Sigma Cat. # T7451) and 1:500 pan-tubulin (Cat. # ATN02). Detection with the secondary antibodies of IRDye 680RD Donkey anti-goat (LiCOR Cat. # 926-68074) and IRDye 800CW Donkey anti-mouse (LiCOR Cat. # 926-32212).

References

1. Barra H.S., et al. (1974) *Biochem. Biophys. Res. Commun.* 60:1384-1390.
2. Eshun-Wilson L., et al. (2019) *PNAS* 116:10366-10371.
3. Dompierre et al. (2007) *J . Neurosci.* 27:3571-3583.
4. Reed et al. (2006) *Curr. Biol.* 16:2166-2172.
5. Nekooki-Machida and Hagiwara. (2020) *Med Mol Morphol.* 53:191–197.

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