V. 1.0

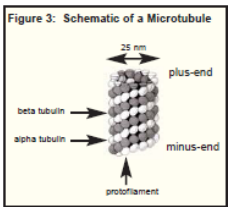
Fungal Tubulin
Isolated from *Agaricus bisporus*
Cat. # F001

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

About Tubulin

Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called α and β tubulin. The two proteins are encoded by separate genes, or small gene families, whose sequences are highly conserved throughout the eukaryotic kingdom.

Tubulin polymerizes to form structures called microtubules (MTs). When tubulin polymerizes it initially forms protofilaments, MTs consist of 13 protofilaments and are 25 nm in diameter. Each µm of MT length is composed of 1650 heterodimers (2). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 3). Tubulin can polymerize from both ends *in vitro*, however, the rate of polymerization is not equal. It has therefore become the convention to call the rapidly polymerizing end the plus-end and the slowly polymerizing end the minus-end. *In vivo* the plus-end of a microtubule is distal to the microtubule organizing center.



The intrinsic ability of pure tubulin to polymerize *in vitro* is very much a function of the experimental conditions. For example, one can manipulate the polymerization reaction to yield microtubules of a particular mean length distribution or create conditions under which tubulin will not polymerize significantly until an enhancer component, such as a polymerization stimulating drug or protein, is added. The propensity of tubulin subunits to assemble into microtubules is dependent on their affinity for microtubule ends (termed critical concentration [CC]). In order to achieve polymerization the CC needs to be less than the total tubulin concentration. At concentrations above the CC, tubulin will polymerize until the free subunit concentration is equal to the CC value.

Material

Tubulin protein has been isolated from the mushroom, *Agaricus bisporus*, and purified by anion exchange chromatography. Tubulin consists of one α and one β isotype. Each isotype is 55 kDa in size, as demonstrated by SDS-PAGE analysis. Typically, the molar equivalent of tubulin is defined as the heterodimer, which has a molecular weight of 110 kDa. Tubulin is supplied as a white lyophilized powder in the heterodimeric form.

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C. The protein should be reconstituted to 1.1 mg/ml as described in the Biological Activity Assay section. Re-freezing of reconstituted protein is not recommended as significant denaturation can occur.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue-stained protein from 4-20% SDS-PAGE. F001 is determined to be >90% fungal tubulin (molecular weight of 55 kDa).

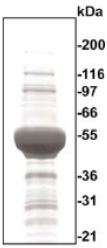


Figure 1. F001 Protein Purity Determination.
A 20 µg sample of F001 protein was separated by 4-20% SDS-PAGE and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band.

Biological Activity Assay

The biological activity of F001 can be assessed by a tubulin polymerization assay. The polymerization of tubulin into microtubules can be detected by measuring the optical density at 340 nm (see Figure 2). Under the experimental conditions defined below, a 100 µl solution of F001 at 1.0 mg/ml in a 1/2 area 96-well plate should result in a change in optical density of >0.25 within 1 h at 30°C.

Note: When measuring the optical density of samples in a microtiter plate using a spectrophotometer, the readings are taken from the top of the plate and thus the pathlength is determined by the reaction volume. Given that a longer pathlength yields an optimal signal, it is important to either use a larger volume or plates with reduced well area. We highly recommended the use 1/2 area well plates or 384 well plates for a longer pathlength and thus optimal signal.

Reagents

1. Fungal Tubulin protein (Cat. # F001)
2. GTP, 100 mM solution (Cat. # BST06)
3. 10% PEG Buffer (10% PEG 10,000 (Fluka), 80 mM PIPES, pH 7.0, 0.5 mM EGTA, 2.0 mM $MgCl_2$, 5% glycerol (included)).
4. PEM+5% glycerol (80 mM PIPES, 0.5 mM EGTA, 2.0 mM $MgCl_2$, 5% glycerol)

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

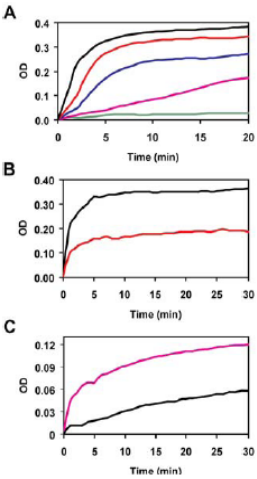
Equipment

1. Temperature regulated spectrophotometer set on kinetic mode at 340 nm, one reading every min for 1 h.
2. Half area 96 well plate (Corning Cat. # 3696) or a standard 384 well plate.

Method

1. Place a 1/2 area 96 well plate (Corning Cat. # 3696) or a 384 well plate into a plate reader that is set to 30°C.
2. Dilute 10% PEG to 7% using PEM+5% glycerol
3. Supplement PEM+5% glycerol and 7% PEG solutions with fresh GTP to 1 mM. Keep on ice.
4. Centriguge the vial of F001 to collect the lyophilized material in the bottom of the tube.
5. Embed the vial of F001 into ice.
6. Resuspend to 2.2 mg/ml by adding 113 µl of ice-cold PEM+5% glycerol.
7. Allow solution to sit on ice for 30 min.
8. Prepare the chemicals to be tested at a 10x concentration in 3.5% PEG solution. Up to 10% DMSO can be added to this mixture from the original compound stock in DMSO. Keep at room temperature until use.
9. Pipette either 11 µl of diluted chemicals to a warmed 1/2 area 96-well plate or 3.5 µl into the bottom of a 384 well plate.
10. Dilute F001 to 1.1 mg/ml by adding 113 µl of 7% PEG solution.
11. Pipette either 110 µl of diluted F001 into each well of a 96 well plate, or 35 µl into a 384 well plate.
12. Immediately start the plate reader. The program should take readings once every min at 340 nm and 30°C for 30 min. You do not need to designate a blank well. All wells can be individually blanked at the beginning of the assay or data can be processed after completion of the experiment using Excel (inquire to tservice@cytoskeleton.com for a suitable Excel template).
13. Figure 2B shows the results of polymerizing under the conditions described above. To test microtubule stabilizers such as Taxol, follow the protocol described above but replace the 7% PEG solution with 5% PEG, as was done in Figure 2C. It should be noted that you may wish to optimize your particular assay by altering the concentration of PEG in all buffers (see Figure 2A)

Figure 2. Tubulin Polymerization Assays.



A. PEG Dose-Response. Polymerizations were carried out in G-PEM (1 mM GTP in PEM) supplemented with 5% glycerol and decreasing concentrations of PEG; 4.0% (black), 3.5% (red), 3.0% (blue), 2.5% (pink), 0% (green). Reactions were allowed to progress for 20 min at 30°C, with optical density readings (OD340) taken every min.

B. Decreased Polymerization by Rhizoxin In the Presence of 3.5% PEG. Polymerizations were performed the absence (black) or presence (red) of 10 µM Rhizoxin using 3.5% PEG in G-PEM+%5 glycerol as the buffer. Reactions were allowed to progress for 30 min at 30°C, with optical density readings (OD340) taken every min.

C. Enhanced Polymerization by Taxol in the Presence of 2.5% PEG. Polymerizations were carried out in the presence (pink) or absence (black) of 10 µM taxol in G-PEM+%5 glycerol supplemented with 2.5% PEG. Reactions were allowed to progress for 30 min at 30°C, with optical density readings (OD340) taken every min.

Product Uses

- Recommended for IC50 & EC50 determinations for fungal-specific tubulin ligands.
- Recommended for examining interactions of proteins with tubulin.

References

1. Shelanski ML, et al. 1973. Proc. Natl. Acad. Science USA. 70: 765-768
2. Amos, LA. & Klug A. 1974. J. Cell Sci. 14: 523-530.

Product Citations/Related Products

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