Soybean Tubulin V. 3.0

Isolated from Soybean Var. 0127562 (Garst Inc. Agripro Round-Up Ready)

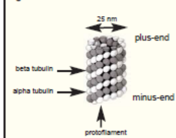
Cat. # TP005

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

About Tubulin

Tubulin is a heterodimer consisting of two 55 kDa subunits called α and ß tubulin. Tubulin polymerizes into structures called microtubules (MTs). MTs are highly ordered and have an intrinsic polarity (see Fig. 3). Tubulin can polymerize from both ends *in vitro*; however, the rate of polymerization is not equal. It has 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 MT is distal to the MT organizing center. The intrinsic ability of pure tubulin to polymerize *in vitro* is very much a function of the experimental conditions. For example, the polymerization reaction can be altered to yield MTs of a particular mean length distribution or create conditions under which tubulin will not polymerize significantly until an enhancer component, such as a stimulating drug or protein, is added. The propensity of tubulin subunits to assemble into MTs is dependent on their affinity for MT 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 tubulin concentration is equal to the CC value.

Figure 3: Schematic of a Microtubule



We provide tubulins purified from soybean and maize plants. A significant advantage of using tubulins from these sources over animal tubulins is that they are derived from plant cells and are thus a more appropriate model systems for plant research. Tubulins from different tissues vary in the relative abundance of specific isoforms and the nature of post-translational modifications. These tissue type specific variants of tubulin have different biological and biochemical properties. It follows that the development of anti-tubulin ligands would benefit from the use of tubulin species purified from tissues that are relevant to the pathology under investigation. The specificity of ligands for a particular tubulin variant can be determined by performing comparative studies with both plant and neuronal tubulins. We have advanced this concept by developing the Tubulin Ligand Index (TLI) system (patent pending). In this system, IC50 values for inhibitory compounds or EC50 values for stabilizing molecules are determined in polymerization assays using plant and neuronal tubulins. The IC50 or EC50 values for each tubulin variant are analyzed as a ratio (neuronal/plant) and allow for determinations of the relative specificity for each tested compound. TLI values greater than 1.0 indicate that a particular compound is more active on plant tubulin. Conversely, TLI values less than 1.0 suggest that a compound is more specific for neuronal tubulin. Table 1 summarizes data from a study comparing the specificity of several tubulin ligands using the TLI system.

Table 1. Tubulin Ligand Index Values from Studies with Soybean and Neuronal Tubulin.

Ligand	EC50* or IC50* Neuronal Tubulin (uM)	EC50* or IC50* Soybean Tubulin (uM)	Tubulin Ligand Index Ratio (Neuronal/Soybean)
Paclitaxel	0.48	3.60	0.133
Nocodazole	3.40	>100	<0.034
Pendimethalin	>100	3.60	>28
Trifluralin	>100	1.90	>52

Material

Soybean tubulin is isolated from 6 day old soybean germlings of the variety 0127562 (Garst Inc. Agripro Round-Up Ready) using anion exchange chromatography. Tubulin from soybean may be used in all situations where other tubulins have been employed, such as compound screening, motility assays, binding assays, and polymerizaton assays. The advantage of using TP005 over animal tubulins is that TP005 is derived solely from plant cells and thus more accurately portrays the tubuin phenotype of plant cells. TP005 is supplied as a white powder containing 250 µg of soybean tubulin protein. When reconstituted to 2.5 mg/ml with 0.1% Triton X-100 in water the buffer formula is 7.5 mM PIPES pH 7.0, 0.25 mM $MgCl_2$, 0.25 mM GTP, 0.1% Triton X-100, 2.5% Sucrose, and 0.5% Ficoll400.

Storage and Reconstitution

In the lyophilized form TP005 is stable for 6 months when stored at 4°C or -70°C in a desiccator. In preparation for polymerization assays, each vial of TP005 should be placed on ice and reconstituted with 83 µl of ice cold 0.1% Triton X-100 in Milli-Q water and then incubated on ice for 60 min. It is not recommended to store reconstituted protein as the polymerization activity is greatly reduced and the IC50 values are altered.

Purity

Protein purity is determined by running 20 µg of protein on a PAGE-SDS gel, (see Fig. 1) and performing scanning densitometry of the protein band stained with Coomassie Blue from 4-20% SDS-PAGE. TP005 is determined to be greater than 90% Soybean tubulin (molecular weight of 54 kDa).

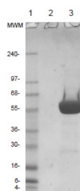


Figure 1. TP005 Protein Purity Determination. A 20 µg sample of TP005 protein was separated by 4-20% SDS-PAGE and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker.

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Biological Activity Assay

The biological activity of TP005 can be assessed by polymerization assays. Tubulin polymerization into MTs can be detected by measuring the optical density (OD) at 340 nm or by fluorescence in the presence of DAPI at excitation and emission wavelengths of 360 nm and 405-450 nm, respectively.

Reagents

1. Soybean Tubulin protein (Cat. # TP005).
2. Triton X-100 solution (0.1% v/v in nanopure water at 4°C).
3. Buffer 1 (5X stock: 300 mM PIPES pH 6.9, 225 µM DAPI).
4. Buffer 2 (5X stock: 60% PEM buffer (v/v), 40% DMSO).
5. PEM buffer: 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA.
6. Paclitaxel (Cat. # TXD01, 2 mM in DMSO).
7. DMSO for Buffer 2 and paclitaxel.
8. DAPI, 10 mM (for fluorescence experiments only).

Equipment

1. Temperature regulated spectrophotometer plate reader set on kinetic mode at 340 nm (for OD experiments) or fluorimeter set to excitation at 360 nm and emission at 405-450 nm (for fluorescence experiments).
2. A low volume 384 well plate (Corning Cat. # 3917) for fluorescence measurements, or a half area 96 well plate (Corning Cat. # 3696) for optical density measurements.

Method 1. Detecting Polymerization by Measuring Fluorescence

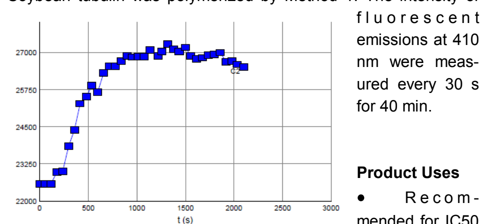
1. Place 384 well plate in fluorimeter set to 30°C.
2. Centrifuge TP005 vial at 14,000xg for 30 sec to collect powder to the bottom of the tube.
3. Place vial on ice and reconstitute with 83 µl of ice-cold 0.1% Triton X-100 in Milli-Q water. Do not pipette vigorously, just allow solution to sit at bottom of the tube.
4. Incubate on ice for 60 min.
5. Prepare test compounds, buffers, and paclitaxel.
6. Dilute the compounds to be tested at 5X the desired final concentration using Buffer 2 (inhibitors) or water (enhancers). Keep at room temperature until use (stable for 4 h).
7. After 60 min on ice, pipette 20 µl of Buffer 1 into tubulin and mix well.
8. Pipette 2 µl of compound solution from Step 6 or Buffer 2 only into each well.
9. Pipette 8 µl of tubulin solution into each well and begin reading fluorescence every 60 s for 40 min (see Fig. 2).
10. Note: if polymerization phase is too steep, then tubulin concentration can be reduced to 1.5 or 2.0 mg/ml in Step 3.

Method 2. Detecting Polymerization by Measuring Optical Density

1. Place 1/2 area 96-well plate into a plate reader set to 30°C.
2. Place TP005 vial on ice and reconstitute with 83 µl of ice-cold 0.1% Triton X-100 in Milli-Q water.
3. Allow solution to sit on ice for 60 min.
4. Prepare test compounds, buffers, and paclitaxel.
5. Dilute the compounds to be tested at 10X the desired final concentration using Buffer 2 (inhibitors) or water (enhancers). Keep at room temperature until use (stable for 4h).
6. Pipette 10 µl of the 10X diluted chemicals into the warmed plate.
7. Add 100 µl of TP005 into each well of the plate.

Immediately start the plate reader, taking readings every min for 1 h. You do not need to designate a blank well. All wells can be individually referenced to AFU or OD = 0 at the beginning of the assay or Vmax data can be processed after completion of the experiment using Excel (inquire to tservice@cytoskeleton.com for a suitable Excel template)

Figure 2. Polymerization of Soybean Tubulin.
Soybean tubulin was polymerized by Method 1. The intensity of fluorescent emissions at 410 nm were measured every 30 s for 40 min.



Product Uses

- Recommended for IC50 & EC50 determinations for plant-specific tubulin ligands.
- Recommended for examining protein interactions with plant-specific tubulin.

Product Citations/Related Products

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