V. 1.0

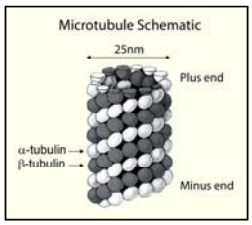
**Tubulin protein from tumor tissue (>95% pure)**
**Source: Caki-1 human tumor grown in mice.**
**Cat. # TM001**
**Lot # 011**
**Upon arrival store at –70°C**

**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.

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 (1). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 1).

**Figure 1. Microtubule Schematic**




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 give 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 upon 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. Because of this parameter, pure tumor tubulin in General Tubulin Buffer (80 mM PIPES, pH 7.0, 2 mM MgCl2, 0.5 mM EGTA and 1 mM GTP) will not polymerize significantly at concentrations below 1 mg/ml. If, however, one adds a polymerization enhancer such as 10% glycerol to 2mg/ml tumor tubulin, polymerization efficiency will approach 90% polymer mass at 37°C after 15-20 minutes. Tubulin polymerization is also a temperature sensitive event, optimal polymerization occurs at 37°C.

**Material**

Tubulin protein has been purified from Caki-1 tumor tissue by an adaptation of the method of Davis et al. (2), using anion exchange chromatography followed by a cycle of polymerization/ depolymerization. TM001 is supplied in 250 µg aliquots in a lyophilized format in 10 mM Na-PIPES buffer pH 6.9, 0.25 mM $MgCl_2$, 0.1 mM GTP, 5% sucrose and 1% Ficoll-400K. Tubulin consists of a heterodimer of one alpha and one beta isotype, each tubulin isotype is 55 kDa in size, SDS-PAGE analysis shows tubulin running as a 55 kDa species (see Figure 2). Typically, the molar equivalent of tubulin is defined as the heterodimer which has a molecular weight of 110 kDa.

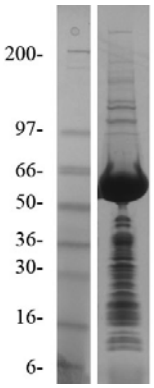



**Figure 2. Purity Analysis of Tubulin Protein.**

A 100 µg sample of TM001 protein was separated by electrophoresis in a 4-20% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark 12). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band. Purity of TM001 was determined to be >90%.

**Storage**

On arrival, it is recommended that TM001 is stored in a desiccated chamber at <10% at 4°C, where it is stable for 6 months. After reconstitution in 50 µl of ice cold freshly made G-PEM plus 10% glycerol and 0.05% Triton X-100, the protein is stable for 1h on ice. If aliquots are needed for future experiments then resuspend to 4mg/ml with 62.5 µl of the same buffer and snap freeze experimental sized aliquots in liquid nitrogen and store at -70°C. **Aliquots of T234S MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this results in significant loss of activity**. Likewise defrosting tubulin solution should be rapid by placing the tube in room temperature water for 1min then transfer to ice.

**Biological Activity Assay**

The biological activity of TM001 is assessed by a tubulin polymerization assay. The ability of tubulin to polymerize into microtubules can be followed by observing an increase in fluorescence using 10 µM Dapi in the G-PEM buffer plus 10% glycerol with 2mg/ml tubulin (see Figure 3). Under these experimental conditions the fluorescence will increase by 15-20% over 15 minutes at 37°C (see Figure 3). The assay volume is 10 µl and a low volume 384 well plate is used (Corning # 3676).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**Reagents**

1. Tubulin protein (Cat. # TM001)
2. GTP stock (100 mM) (Cat. # BST06)
3. Tubulin Buffer (G-PEM); 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA and 1 mM GTP (made fresh or from frozen stock)

**Reagents**

1. Temperature regulated fluorescence plate reader at 37°C on kinetic mode at Excitation 360 nm, and Emission 410 or 420nm.
2. Low volume 384-well plate (Corning Cat # 3676)

**Method**

1. Place one TM001 vial on ice.
2. Resuspend in 125 µl of G-PEM plus 10% glycerol and 0.05% Triton X-100.
3. Prepare compounds for screening at 5x concentration in PEM plus 5% DMSO.
4. Pipette 2 µl compound into each well of a pre-warmed 37°C plate.
5. Pipette 10 µl of tubulin solution into each well and start the plate reader protocol. Measure tubulin polymerization by taking readings every 30 seconds for 30 min.
6. Figure 3 shows the results of polymerizing TM001 under the conditions described above.

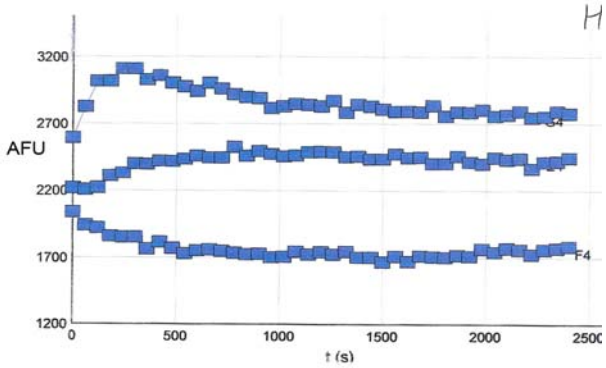


**Figure 3. Tubulin Polymerization Assay**

Polymerizations were carried out as indicated in the Method section. Polymerization was started by incubation at 37°C and followed by fluorescence read at 360nmEx / 410nmEm. Under these conditions the control reaction reached a maximal fluorescence after 1000 seconds (middle curve). Top curve contains 10 µM taxol whereas the lower curve contains 10 µM vinblastine.

**Important Technical Notes when Working with Tubulin protein**

1. Any buffer containing GTP should be kept on ice and used within 1-2h after addition of GTP as GTP will hydrolyze over time. Unused GTP supplemented buffer should be discarded.
2. Tubulin is a labile protein and should be used immediately after thawed or snap frozen into appropriate aliquots (see Storage section). Freeze/thaw cycles should be avoided. Keep tubulin on ice prior to beginning the polymerization reaction.
3. Temperature is an extremely important parameter for tubulin polymerization. Temperatures cooler than 37°C will significantly decrease the rate and final $OD_{340nm}$ reading of a polymerization reaction. If tubulin is aliquoted into a cool plate (or room temperature plate) there will be a much longer nucleation phase (Figure 3).
4. Polymerization conditions can be altered to optimize a given assay requirement. For example, to examine polymerization enhancers such as taxol, it is recommended to reduce the tubulin concentration to 1 3 mg/ml and polymerize in buffer without glycerol. These conditions will result in a very slow and shallow polymerization curve for the “no compound” control. In this case, efficient polymerization is achieved by addition of an enhancer such as taxol (5 - 10 µM final concentration).

**Product Uses**

- IC50 & EC50 determinations for anti-tubulin ligands.
- Characterization of tubulin binding proteins.

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**References**

1. Amos, LA. & Klug A. 1974. J. Cell Sci. 14: 523-530.
2. Davis A. et al. 2010. Mets. Cell Biol., 97, Ch 18, p331-351.

**Product Citations/Related Products**

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