V. 2.0

HeLa Cell Tubulin
Isolated from HeLa S3 cell line
Cat. # H001

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

About Tubulin

Tubulin is heterodimer consisting of two 55 kDa subunits called α and β tubulin. Tubulin polymerizes into structures called microtubules (MTs). MTs are highly ordered and have an intrinsic polarity (Fig. 1). 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 1. Microtubule Schematic

We provide tubulins purified from HeLa cervical carcinoma cells and MCF-7 breast cancer epithelium cells. A significant advantage of using tubulins from HeLa or MCF-7 cell lines over neuronal tubulins is that they are derived from actively dividing human cancer cells and are thus more appropriate model systems for cancer research. Tubulins from different tissue 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 cancer cell 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 cancer cell and neuronal tubulins. The IC50 or EC50 values for each tubulin variant are analyzed as a ratio (neuronal/cancer cell) 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 cancer cell 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 Cancer Cell and Neuronal Tubulin

Ligand	EC50/IC50 Value			TLI Value	
	Neuronal	MCF-7	HeLa	MCF-7	HeLa
Paclitaxel	0.48	0.51	1.04	0.94	0.46
Docetaxel	0.47	0.34	0.41	1.38	1.15
10-Deacetyl taxol	3.71	4.20	30.00	0.88	0.12
Vinblastine	1.10	1.21	2.83	0.91	0.39
Vincristine	1.58	nd	2.25	na	0.70
Colchicine	4.10	4.60	3.10	0.89	1.32
Nocodazole	3.40	3.20	3.20	1.06	1.06
Mebendazole	3.98	14.80	25.00	0.27	0.16
MF708	3.54	nd	1.91	na	1.85

Material

Hela Cell Tubulin is isolated from the Hela S3 cell line using anion exchange chromatography. HeLa cells are a commonly used model system to study many aspects of tumor cell biology. Tubulin from HeLa cells may be used in all situations where mammalian brain tubulin has been employed, such as drug screening, motility assays, and polymerization assays. The advantage of using H001 is that, unlike neuronal tubulins, it is derived from actively dividing carcinoma cells and thus more accurately portrays a tumorigenic phenotype. H001 is supplied as a white powder containing 250 µg of HeLa cell tubulin protein lyophilized in 80 mM PIPES pH 7.0, 1 mM $MgCl_2$, 0.5 mM EGTA, 0.1 mM GTP, 1.0 mM DTT, 5% Sucrose, and 1% Ficoll400.

Storage and Reconstitution

In the lyophilized form H001 is stable for 6 months when stored at 4°C. In preparation for polymerization assays, each vial of H001 should be reconstituted to 2.0-2.4 mg/ml with ice cold G-PEM (80 mM PIPES pH 7.0, 0.5 mM EGTA, 1.0 mM $MgCl_2$, and 1.0 mM GTP) supplemented with 20% glycerol. H001 can also be frozen in small aliquots after being resuspended to 5 mg/ml in G-PEM supplemented with 20% glycerol. Samples should then be drop frozen in liquid nitrogen and stored at -70°C.

Purity

Protein purity is determined by scanning densitometry of protein stained with Coomassie Blue from 4-20% SDS-PAGE (Fig. 2). H001 is determined to be greater than 90% HeLa cell tubulin (molecular weight of 55 kDa).

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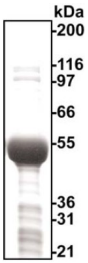

Figure 2. H001 Protein Purity Determination.
A 20 µg sample of H001 protein was separated by 4-20% SDS-PAGE and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker.

Biological Activity Assay

The biological activity of H001 can be assessed by polymerization assays (Fig. 3). 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. HeLa Cell Tubulin protein (Cat. # H001)
2. GTP, 100 mM solution (Cat. # BST06), keep on ice.
3. PEMT = 0.1%(v/v) Triton X-100, 80 mM PIPES, 0.5 mM EGTA, 2.0 mM $MgCl_2$. Keep on ice.
4. PEM80G = 80%(v/v) glycerol, 80 mM PIPES, 0.5 mM EGTA, 2.0 mM $MgCl_2$. Keep on ice.
5. DAPI, 10 mM (for fluorescence method 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. Half area 96 well plate (Corning Cat. # 3696) for OD or 384 well plate (Corning Cat. # 3676) for fluorescence measurements.

Method 1. Detecting Polymerization by Measuring Fluorescence

1. Place a low volume 384 well plate in fluorimeter set to 37° C. Use a kinetic protocol with 360Ex/405Em and readings every 30s for 20min.
2. Supplement 1 ml of ice cold PEMT with 1.3 mM GTP(13 µl of 100 mM stock) and 13 µM DAPI (1.3 µl of 10 mM stock) to make GPEMTD.
3. Resuspend H001 to 2.5 mg/ml by adding 100 µl of GPEMDT.
4. Incubate on ice for 30 min.
5. Dilute the compounds to be tested at 5X the desired final concentration using PEM+10% DMSO. Keep at room temperature until use.
6. Pipette 33 µl of PEM80G into each vial of H001 and pipette up and down to mix.
7. Pipette 2 µl of each compound into duplicate or triplicate wells.
8. Pipette 8 µl of H001 into each well and immediately begin reading fluorescence every 30 s for 20 min.

Method 2. Detecting Polymerization by Measuring Optical Density

1. Place 1/2 area 96-well plate into a plate reader set to 37°C.
2. Supplement PEM+0.1% (v/v) Triton X-100 with 1 mM GTP to make GPEMT.
3. Resuspend H001 to 2.5 mg/ml by adding 100 µl of GPEMT.
4. Incubate on ice for 30 min.
5. Dilute the compounds to be tested at 10X the desired final concentration using PEM+10% DMSO. Keep at room temperature until use.
6. Pipette 10 µl of the 10X diluted chemicals into the warmed plate.
7. Pipette 33 µl of PEM80G into each vial of H001 and pipette up and down to mix.
8. Add 100 µl of H001 into each well of the plate.
9. 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 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).

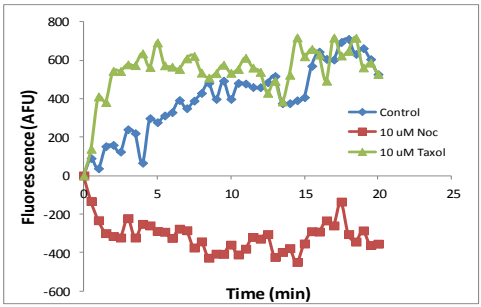

Figure 3. Polymerization of HeLa Cell Tubulin in the presence of nocodazole and taxol.
HeLa cell tubulin polymerization assays were performed in a 10 µl reaction using the fluorescence method in the presence of 10 µM nocodazole (red), a MT inhibitor, and 10 µM taxol (green), a MT enhancer. The intensity of fluorescent emissions at 405 nm were measured every 30 s for 20 min.

Product Uses

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

Product Citations/Related Products

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