V. 1.0

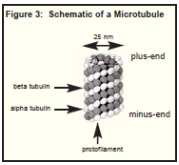
MCF-7 Cell Tubulin
Isolated from MCF-7 cell line
Cat. # H005

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 (see Figure 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 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, the polymerization reaction can be altered 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 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 tubulin concentration is equal to the CC value.

Figure 3: Schematic of a Microtubule



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

MCF-7 Cell Tubulin is isolated from the MCF-7 breast cancer cell line using anion exchange chromatography. MCF-7 cells are a commonly used model system to study many aspects of tumor cell biology. Tubulin from MCF-7 cells may be used in all situations where bovine brain tubulin has been employed, such as drug screening, motility assays, and polymerizaton assays. The advantage of using H005 is that, unlike neuronal tubulins, it is derived from actively dividing carcinoma cells and thus more accurately portrays a tumorigenic phenotype. H005 is supplied as a white powder containing 250 µg of MCF-7 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 H005 is stable for 6 months when stored at -70°C. In preparation for polymerization assays, each vial of H005 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. H005 can also 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. H005 is determined to be greater than 90% MCF-7 cell tubulin (molecular weight of 55 kDa).

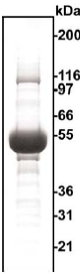


Figure 1. H005 Protein Purity Determination.
A 20 µg sample of H005 protein was separated by 4-20% SDS-PAGE and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker.

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Biological Activity Assay

The biological activity of H005 can be assessed by polymerization assays. Tubulin polymerization into microtubules 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. MCF-7 Cell Tubulin protein (Cat. # H005)
2. GTP, 100 mM solution (Cat. # BST06)
3. PEM+20% glycerol (80 mM PIPES, 0.5 mM EGTA, 2.0 mM $MgCl_2$, 20% (v/v) glycerol)
4. 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. 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 Optical Density

1. Place 1/2 area 96-well plate into a plate reader set to 37°C.
2. Supplement PEM+20% (v/v) glycerol with GTP to 1 mM (G-PEM+20% glycerol).
3. Resuspend H005 to 2.0 mg/ml by adding 125 µl of G-PEM+20% glycerol.
4. Allow solution to sit on ice for 30 min.
5. Dilute the compounds to be tested at 10× the desired final concentration using PEM+20% glycerol. Keep at room temperature until use.
6. Pipette 10 µl of the 10× diluted chemicals into the warmed plate.
7. Add 100 µl of H005 into each well of the plate.
8. 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 (email tservice@cytoskeleton.com for a suitable Excel template).

Method 2. Detecting Polymerization by Measuring Fluorescence

1. Place 384 well plate in fluorimeter set to 37°C.
2. Supplement PEM+20% glycerol with GTP to 1 mM and DAPI to 10 µM.
3. Resuspend H005 to 2.4 mg/ml by adding 104 µl of PEM+20% glycerol supplemented with GTP and DAPI. Allow solution to sit on ice for 30 min.
4. Dilute the compounds to be tested at 5× the desired final concentration using PEM+20% glycerol supplemented with GTP and DAPI. Keep at room temperature until use.
5. Pipette 2 µl of each compound into duplicate or triplicate wells.
6. Put plate back in reader and warm for 1 min.
7. Pipette 9 µl of H005 into each well and begin reading fluorescence every 30 s for 40 min.

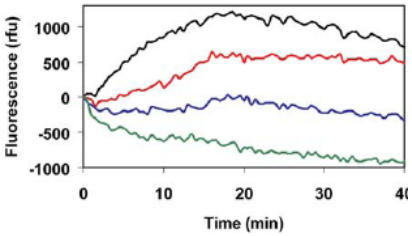

Figure 2. Polymerization of MCF-7 Cell Tubulin In Response to Vinblastine.
MCF-7 cancer cell tubulin polymerizations were carried out in the presence Vinblastine, a microtubule destabilizing agent. H005 was reconstituted in G-PEM +20% glycerol supplemented with DAPI (see Biological Activity Assay) and then incubated at 37°C for 40 min in the absence (black) or presence of 1.0 µM (red), 3.0 µM (blue), or 10 µM (green) Vinblastine. The intensity of fluorescent emissions at 405 nm were measured every 30 s for 40 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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