V. 2.0

Microtubules
(Taxol Stabilized and Lyophilized)
Cat. # MT002

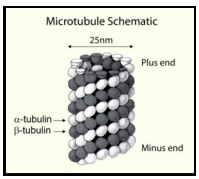
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

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. Consequently, tubulin isolated from porcine brain tissue is highly homologous to tubulin isolated from the majority of eukaryotic sources. This fact results in the technical benefit that porcine tubulin can be used to assay proteins originating from many diverse species.

Tubulin polymerizes to form structures called microtubules (MTs). When tubulin polymerizes it initially forms protofilaments. Microtubules consist of 13 protofilaments and are 25 nm in diameter. Each µm of microtubule length being composed of 1650 heterodimers (1). Microtubules are highly ordered fibers 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 of a microtubule and the slowly polymerizing end the minus-end. *In vivo* the plus end of a microtubule is distal to the microtubule organizing center.

Microtubules (MTs) serve as a substrate for kinesin and dynein motor proteins as well as contributing to the shape and division of cells by the interaction with anchoring proteins. Kinesins operate by utilizing the energy of ATP to hydrolysis, an activity that is greatly enhanced in the presence of MTs. A MT activated kinesin ATPase assay is therefore used as a test for the biological activity of pre-formed microtubules, Cat. # MT002. This substrate is particularly useful for MT stimulated Kinesin ATPase assays that are used to develop novel anti-mitotic compounds, because the MT product is highly quality controlled we have achieved batch to batch variability of less than 5%.

Material

Stablilized microtubules (MTs) are supplied as a white lyophilized powder. Microtubules have been prepared from tubulin protein purified from porcine brain that is greater than 99% pure. The stringently controlled quality control of the MTs provide highly reliable and reproducible results in assays that require MT substrates (2). The average MT length in this product is 2 µm.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 1 year. Lyophilized protein can also be stored dessicated at –70°C.

The reconstitution instructions outlined below have been optimized for a MT activated kinesin ATPase assay. The MTs can be resuspended to any desired concentration and in other buffer as long as taxol is included. If the MTs are to be frozen into aliquots for long term storage after reconstitution then a 5 mg/ml MT concentration is recommended (1 mg/ml can be frozen but will have a shorter shelf life). If you plan to use a different buffer from the one shown we recommend calling technical service to check on compatibility.

Reconstitute as follows:

1. Warm appropriate amount of PM buffer to room temperature to resuspend MTs to 1 mg/ml. (PM buffer = 15 mM PIPES pH 7.0, 1 mM $MgCl_2$).
2. Prepare a 2 mM taxol stock (Cat. # TXD01)in anhydrous DMSO and add 100 µl per 10 ml room temperature PM buffer. This is now MT resuspension buffer. NOTE: If is important to make sure that the PM buffer is at room temperature as taxol will precipitate out of solution if added to cold buffer.
3. Add MT resuspension buffer to the lyophilized MTs to a concentration of 1 mg/ml. Mix gently.
4. Leave the MTs at room temperature for 10-15 minutes with occasional gentle mixing.
5. The MTs are now ready to use. They are at a mean length of 2 µm.
6. The microtubules will be stable for 2-3 days at room temperature, although it should be noted that the mean length distribution will increase over time.
7. Any microtubules that are not used can be snap frozen in convenient aliquots. NOTE: Liquid nitrogen must be used to snap freeze MTs. Stable at –70°C for 3 months.

Biological Activity Assay

MTs are tested in a MT activated Enzyme Linked Inorganic Phosphate ATPase Assay (the ELIPA™ Assay). The assay is available in kit form from Cytoskeleton, inc. (Cat. # BK060) (2). The motor protein used in this assay is Kinesin Heavy Chain motor domain (KHC, Cat.# KR01). The assay is performed as follows:

Reagents

1. Recombinant Kinesin Heavy Chain motor domain (Cat. # KR01)
2. ELIPA Reaction Buffer (15 mM PIPES, 5 mM $MgCl_2$, pH 7.0)
3. ELIPA Reagent 1 (1 mM 2-amino-6-mercapto-7-methylpurine riboside)
4. ELIPA Reagent 2 (0.1 U/µl purine nucleoside phosphorylase)
5. ATP,100 mM stock (Cat. # BSA04)
6. Taxol (Cat. # TXD01, 2 mM stock)

Phone: (303) 322.2254 **Fax:** (303) 322.2257
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Equipment

1. Kinetic Spectrophotometer capable of measuring absorbance at 360 nm (bandwidth < 10 nm) for 96 well plates.
2. 96 well plate
3. Rotator or rocker at room temperature

Method

1. Resuspend Kinesin Heavy Chain motor domain protein (Cat. # KR01) to 0.25 mg/ml in ELIPA reaction buffer.
2. Mix the following components in the order shown below:

MT ELIPA MIX	
ELIPA Reaction buffer (room temperature)	1 ml
Taxol stock	10 µl
MT002 (resuspended to 1 mg/ml as outlined)	80 µl
ELIPA Reagent 1	240 µl
ELIPA Reagent 2	12 µl

3. Agitate the MT ELIPA MIX for 5 minutes on a room temperature rotator or rocker.
4. Add the following components to a 96 well plate as follows:

Well designation	MT ELIPA MIX (µl)	Kinesin protein (µl) (Cat. # KR01)
A1	300	0
B1	300	4
C1	300	4
D1	300	4
E1	300	4

5. Start the reactions by adding ATP to 0.6 mM final concentration using a multichannel pipettor.
6. Immediately read the reactions on a kinetic spectrophotometer set at 360 nm wavelength. It is recommended to take readings once every 30 seconds for a reaction time of 20 minutes.
7. Under these conditions kinesin has a microtubule activated ATPase Vmax activity of 10,000 ATP hydrolyzed per minute per mg of kinesin (see Figure 2).

Results

Reactions that were minus MTs or minus KHC did not show any ATP hydrolysis (see Figure 2). In all reactions containing MTs and KHC the MTs were shown to significantly stimulate KHC ATPase activity (Figure 2).

The reproducibility of microtubule batches from bovine and porcine sources was also examined as part of this quality control. Figure 3 shows MT activated kinesin ATPase activity of microtubules from either bovine (Cat. # MT001) or porcine (Cat. # MT002) sources. Batch to batch reproducibility is >95%.

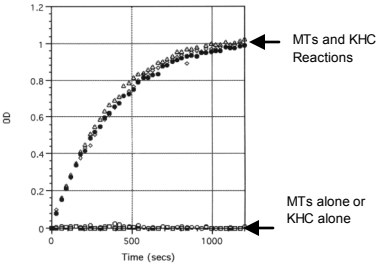

Figure 2: MT Activated Kinesin ATPase Reaction: *Reactions were performed as outlined in the Methods section. Each reaction contains 47 nm kinesin (Cat. # KR01), 0.66 µM pre-formed microtubules (Cat. # MT002), 0.2 mM ELIPA Reagent 1, 0.3 U ELIPA Reagent 2, 20 µM taxol, 15 mM PIPES pH 7.0, 5 mM $MgCl_2$, 0.6 mM ATP. Triplicate reactions were measured in a SpectroMax 250 (Molecular Devices) set in kinetic mode and 360 nm wavelength. Readings were taken once every 30 s for a total reaction time of 20 min. The ATPase rate for KHC was measured at 1,000 nm ATP hydrolyzed/min/mg of KHC.*

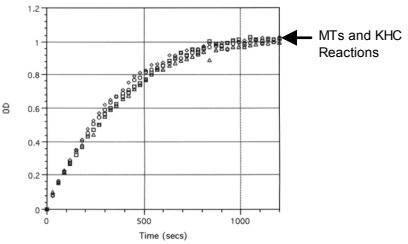

Figure 3: Repoducibility of ATPase activity between MT001 (bovine) and MT002 (porcine) microtubules: *MT002 Lot 001 (circles and squares) and MT002 Lot 017 (diamonds and triangles) were compared for MT activated kinesin ATPase activity. Both reaction conditions were identical to those described in Figure 2, except for the source of microtubules used.*

Product Uses

- Pre-formed MTs can be used in all experiments requiring MTs as substrates. These include kinesin MT activated ATPase assays, microtubule binding assays and motor motility assays.

References

1. Amos, LA. & Klug A. 1974. *J. Cell Sci*. **14**: 523-530.
2. Funk, C.J. et al. 2004. Development of High Throughput Screens for Discovery of Kinesin ATPase modulators. *Analytical Biochemistry*. **329**: 68-76.

Product Citations / Related Products

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