

Product Uses

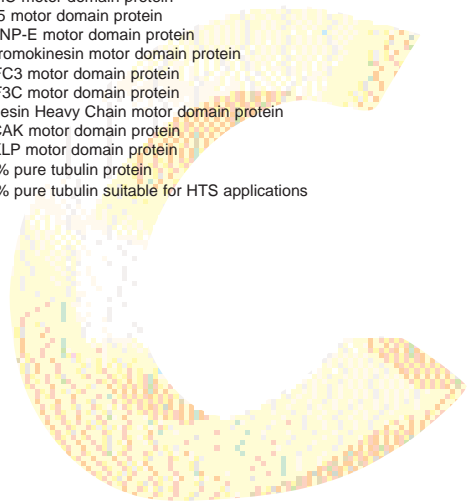
Pre-formed MTs can be used in all experiments requiring MTs as substrates. These include kinesin MT activated ATPase assays, microtubule binding assays and motor motility assays.

References

Funk, C.J. et al. 2003 submitted Development of High-Throughput Screens for Discovery of Kinesin ATPase modulators.

Related Products and Uses

BK051	General ELIPA Assay (general ELIPA ATPase / GTPase reagent)
BK060	Kinesin ELIPA Assay Kit (96 assays)
BM01	BimC motor domain protein
EG01	Eg5 motor domain protein
CP01	CENP-E motor domain protein
CR01	Chromokinesin motor domain protein
KC01	KIFC3 motor domain protein
KF01	KIF3C motor domain protein
KR01	Kinesin Heavy Chain motor domain protein
MK01	MCAK motor domain protein
MP01	MKLP motor domain protein
TL238	99% pure tubulin protein
HTS02	97% pure tubulin suitable for HTS applications



Microtubules

(Taxol Stabilized & Lyophilized)

Cat.# MT002-A 4 x 500 ug

Lot# 003 Store desiccated at 4°C or at -70°C

Material

Stabilized microtubules (MTs) are supplied as a lyophilized powder. Each vial contains 500 µg of tubulin protein. Microtubules have been prepared from porcine tubulin protein that is greater than 99% pure. The stringently quality controlled MTs provide highly reliable and reproducible results in assays that require MT substrates. The average MT length in this product is 2 µm.

Storage and Reconstitution

The lyophilized MTs are stable for one year when stored at 4°C in a desiccant and the humidity is kept below 10%. Alternatively, the lyophilized MTs can be stored at -70°C and are also stable for one year.

Reconstitution

The reconstitution conditions outlined below have been optimized for a MT activated kinesin ATPase assay. The MTs can be resuspended to any desired concentration and in other buffers 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 3 mls of PM buffer to room temperature (15 mM Pipes pH 7.0, 1 mM MgCl₂).
- 2) Prepare a 2 mM taxol stock in dry DMSO (cat# TXD01) and add 30 µl to the room temperature PM buffer. This is your MT resuspension buffer. NOTE: it 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 500 µl of MT resuspension buffer to the 500 µg of lyophilized MTs and 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 and the tubulin concentration is 1 mg/ml.
- 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 liquid nitrogen in convenient aliquots. NOTE: liquid nitrogen must be used to snap freeze MTs. Stable at -70°C for 3 months.

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Biological Activity Assay

MTs serve as a substrate for kinesin motor proteins. Kinesin motor proteins orchestrate a wide range of kinetic events within a cell. They have been shown to move cargoes such as chromosomes and vesicles along MT tracks. 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 MT002.

MTs are tested in a MT activated Enzyme Linked Inorganic Phosphate ATPase assay (the ELIPATTM assay). The assay is available in kit form from Cytoskeleton Inc. (catalog # BK060). The motor protein used in this assay is kinesin Heavy Chain (KHC). The assay is performed as follows;

Reagents

- 1) ELIPA reaction buffer: 15 mM PIPES pH7.0, 5 mM MgCl₂
- 2) ELIPA Reagent 1: 1 mM 2-amino-6-mercapto-7-methylpurine riboside (MESG)
- 3) ELIPA Reagent 2: purine nucleoside phosphorylase (PNP), 0.1U / ul
- 4) ATP solution: 10 mM made fresh from a 100 mM stock solution
- 5) Taxol (Cat. # TXD01): 2 mM stock in dry DMSO
- 6) Kinesin Heavy Chain (KHC) protein (Cat. # KR01): 0.5 mg/ml
- 7) Pre-formed microtubules (Cat. # MT002)

Equipment

- 1) Kinetic spectrophotometer set to 360 nm. Note: if using a filter based system the filter bandwidth should be <10 nm.

Method

- 1) Set up a 2 ml reaction that contains the following components;

92 nM KHC
660 nM MT002 (prepared as indicated in this profile)
0.2 mM MESG
0.3 U PNP
20 uM taxol
15 mM PIPES pH7.0
5 mM MgCl₂
- 2) Set up two separate 2 ml control reactions as follows
MT Control contains all reaction components above except MTs
KHC Control contains all reaction components above except KHC
- 3) Mix the reactions slowly on a room temperature rotator for 5 minutes.
- 4) Aliquot 300 ul of the KHC reaction into eight wells of a 96 well plate.
- 5) Aliquot 300 ul of the MINUS MT control reactions into eight wells of a 96 well plate.
- 6) Aliquot 300 ul of the MINUS KHC control reactions into eight wells of a 96 well plate.
- 7) Start the reactions by adding 0.6 mM ATP.
- 8) Measure ATP hydrolysis at 360 nm.

Results

Reactions that were minus MTs or minus KHC did not show any ATP hydrolysis (see Figure 1). In all reactions containing MTs and KHC the MTs were shown to significantly stimulate KHC ATPase activity (Figure 1).

The reproducibility of microtubule batches from bovine and porcine sources was also examined as part of this quality control. Figure 2 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 1. MT Activated Kinesin ATPase Reactions

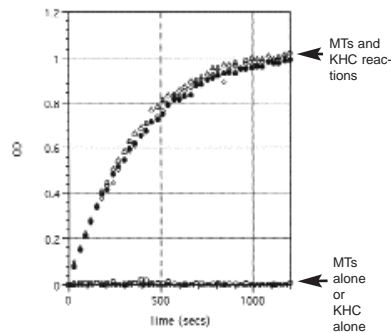


Figure 1. MT Activated Kinesin ATPase Reaction. Reactions were performed as outlined in the methods section. Each reaction contains 47 nM kinesin (Cat. # KR01), 0.66 μ M pre-formed microtubules (Cat. # MT002), 0.2 mM ELIPA Reagent 1, 0.3 units ELIPA Reagent 2, 20 μ M taxol, 15 mM PIPES pH 7.0, 5 mM MgCl₂, 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 seconds for a total reaction time of 20 minutes. The ATPase rate for KHC was measured at 10,000 nmoles ATP hydrolyzed/min/mg of KHC.

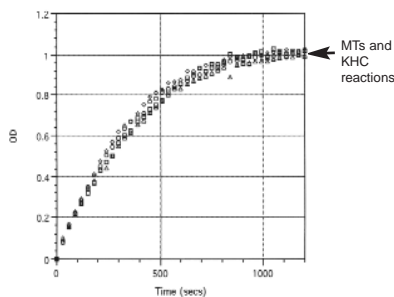


Figure 2. Reproducibility of ATPase activity between MT001 (bovine) and MT002 (porcine) microtubules. MT002 lot 003 (circles & squares) and MT001 lot 017 (diamonds & triangles) were compared for MT activated kinesin ATPase activity. Both reaction conditions were identical to those described in Figure 1, except for the source of microtubules used.