

HTS-Tubulin
(contains 3% MAPs)
Cat. # HTS03-A

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

Material

Tubulin protein has been isolated from porcine brain. The final product contains approximately 97% tubulin and 3% Microtubule Associated Proteins (MAPs). The tubulin is packaged in ASSAY UNITS (see Unit Definition and Table 1). Each Lot of HTS03 is individually quality controlled to determine the exact number of assays per tube. Table 1 shows the minimum guaranteed assays per tube and the actual number of assays available in a particular Lot.

Table 1

HTS03 Size	Minimum Assay Units per tube	Actual Assay Units for this lot	Volume of G-PEM buffer for polymerization (mls)	Amount of tubulin per tube (mg)
HTS03-A	10	10	1.0	4

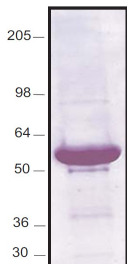
Storage and Reconstitution

The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. To store reconstituted HTS03-A it is recommended to resuspend the protein to 10 mg/ml in ice cold G-PEM buffer (1 mM GTP [add fresh immediately prior to use from a 100 mM stock], 80 mM PIPES, 0.5 mM EGTA, 2 mM MgCl₂, pH 6.9), snap freeze "experiment sized" aliquots in liquid nitrogen and store at -70°C. Reconstituted HTS03 is stable for 6 months at -70°C. Reconstituted HTS03 MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this will result in significant loss of activity.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gel. HTS03 is determined to be 97% tubulin (mol. wt 55 kD) and 3% MAPs (mol. wt 35 - 280 kD), see figure 1.

Figure 1. HTS03 Protein Purity Determination. A 30 µg sample of HTS02 protein was separated by electrophoresis in a 12% 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.



Assay Unit Definition

An ASSAY UNIT is defined as the amount of HTS03 protein needed to achieve a tubulin polymerization signal of OD₃₄₀ of 0.1 - 0.15 in 30 minutes at 37°C in G-PEM buffer. The assay volume is 100 µl and assumes a spectrophotometer pathlength of 0.5 cm. The protein concentration for HTS03 in this assay is approximately 4 mg/ml. NOTE: when using a microtiter plate reading spectrophotometer the readings are taken from the top of the plate and therefore the volume of your reaction will directly influence the pathlength (see Figure 2). Cytoskeleton Inc. highly recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

Biological Activity Assay

The biological activity of HTS03 is assessed by a tubulin polymerization assay. One ASSAY UNIT of tubulin is used for each polymerization assay. An OD₃₄₀ of 0.10 - 0.15 is required to pass quality control. Tubulin polymerization must also be responsive to polymerization enhancers (paclitaxel) and inhibitors (nocodazole) at 5 µM drug concentration (see Vmax values in Figure 2).

Reagents

1. HTS03
2. Paclitaxel (2 mM stock in anhydrous DMSO)
3. Nocodazole (2 mM stock in anhydrous DMSO)
4. G-PEM buffer (1 mM GTP, 80 mM PIPES, 0.5 mM EGTA, 2 mM MgCl₂, pH 6.9)

Equipment

1. Temperature regulated spectrophotometer set on kinetic mode at 340 nm.

Method

1. Place each vial of HTS03 on ice and reconstitute in ice cold G-PEM buffer according to the volumes indicated in Table 1. Note: the GTP should be added from a 100 mM frozen stock immediately prior to use.
2. Leave the protein on ice for 5 - 10 minutes to soften the tubulin protein pellet.
3. The vial of protein should then be mixed well with a pipette to make sure that the protein has resuspended evenly.

4. Tubulin is a labile protein and should be used immediately after resuspension. Keep tubulin on ice prior to beginning the polymerization reaction.
5. For a standard 96 well plate assay, transfer 100 μ l of the resuspended HTS03 (at 4°C) into a microtiter plate that has been pre-warmed to 37°C. Cytoskeleton Inc. highly recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization in this assay.
6. Measure tubulin polymerization by taking readings every 30 seconds at 340 nm and 37°C for 45 minutes to 1 hour. You do not need to designate a blank well, all wells can be individually blanked at the beginning of the assay.
7. Note: Temperature is an extremely important parameter for tubulin polymerization, temperatures cooler than 37°C will significantly decrease the rate and final OD reading of a polymerization reaction.
8. Figure 2 shows the results of polymerizing HTS03 at the recommended dilutions (see Table 1). It should be noted that you may wish to optimize your particular assay by either altering the protein concentration and / or the final reaction volume. Under the reaction conditions described here the assay is responsive to polymerization enhancers such as paclitaxel (0.5 μ M and 5 μ M were tested) and inhibitors such as nocodazole (0.5 μ M and 5 μ M were tested).

Product Uses

- Recommended for primary screens to identify anti-tubulin ligands.
- Production of microtubule substrates for HTS motor assays.

Figure 2: HTS03 Polymerization

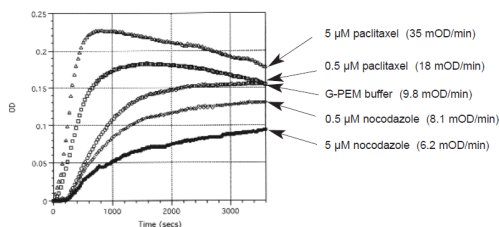


Figure 2: HTS03 Polymerization. Polymerizations were carried out as indicated in the Method section. Paclitaxel and nocodazole were added to wells immediately prior to the addition of HTS03. All reactions contained DMSO at a final concentration of 1%. The V_{max} values for tubulin polymerization is given in parenthesis after each reaction condition, V_{max} values represent 8 data points after each reaction condition, V_{max} values represent 8 data points after each reaction condition and are given in milli-OD units per minute.