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PurSolutions LLC
1203 River Vista Ave
Charlottesville, VA 22901

DATASHEET: LYOPHILIZED TUBULIN (>99%)

Catalog Number: 142001
Source: Bovine Brain
Store Desiccated at 4°C

Background:

Tubulin, a highly conserved cytoskeletal protein, is required for several essential eukaryotic processes including intracellular transport, intercellular signaling, extracellular sensing, cell migration, and cell division. Tubulin (110 kDa) is a heterodimer of α- and β-tubulin (each 55 kDa), and polymerizes into higher order filaments termed microtubules (MTs). MTs measure 25 nm in diameter and have a persistence length of ~2 mm, incorporating ~1650 tubulin subunits per 1 μm. Given the asymmetry of tubulin dimers, MTs have inherent polarity with distinct "+" (β-tubulin exposed) and "-" (α-tubulin exposed) ends. Another critical feature of MTs is their dynamic instability, a consequence of the GTPase activity of tubulin. This property confers force-generating capabilities to MTs that are critical for cell division. For this reason, tubulin is a powerful target for the therapeutic intervention of neoplastic diseases such as cancers.

Material:

Lyophilized Tubulin is isolated by selective precipitation from bovine brain homogenate by an adaptation of the method of Andreu (2007) and lyophilized by an adaptation of the method of Dráberová *et al*. (2010). The resulting product is >99% pure (Figure 1) and polymerization competent (Figure 2). Lyophilized Tubulin is supplied as a white powder with a tubulin-to-powder weight ratio of 1:2. When reconstituted with Milli-Q water to 40 mg/ml, the buffer conditions are 10 mM Sodium Phosphate, 0.5 mM $MgCl_2$, 0.1 mM GTP, and 0.25 M Trehalose, pH 7.0.

Storage and Handling:

Store Lyophilized Tubulin desiccated at 4°C. The product is stable under these conditions for 1 year. Reconstitute Lyophilized Tubulin by resuspending in ice-cold Milli-Q water to 40 mg/ml (note that 1 mg tubulin = 3 mg powder). Add EGTA and $MgCl_2$ to 1 mM each, and clarify the solution at 14,000 rpm for 1 minute at 4°C. Measure the protein concentration and dilute to 20 mg/ml with Tubulin Phosphate Buffer (Cat. No. 142002; 10 mM Sodium Phosphate, 1 mM EGTA, and 1 mM $MgCl_2$, pH 7.0). Reconstituted Lyophilized Tubulin can be buffer-exchanged or cycled as desired, as well as frozen in liquid Nitrogen and stored at -80°C. Avoid repeated freeze-thaw cycles.

Activity:

When supplemented with guanosine (GTP or GMPCPP) and warmed to 37°C, Lyophilized Tubulin will polymerize into MTs when above its critical concentration. The recommended tubulin concentration for ensuring polymerization is 2 mg/ml.

Uses:
Lyophilized Tubulin is supplied for use in cell-free experimental systems including:

- structural analysis by X-ray crystallography and electron microscopy
- drug discovery by high-throughput screening
- *in vitro* biochemical and biophysical approaches

Polymerization Protocol:
Dilute reconstituted Lyophilized Tubulin to 2 mg/ml with Tubulin Phosphate Buffer (Cat. No. 142002; 10 mM Sodium Phosphate, 1 mM EGTA, and 1 mM $MgCl_2$, pH 7.0) and supplement with 1 mM each DTT and guanosine (GTP or GMPCPP). Incubate on ice for 5 minutes, then transfer to a 37° C water bath for 1 hour. If polymerized with GMPCPP or protected with Taxol, the resulting MTs will be stable at room temperature for several days. Do not place polymerized MTs on ice.

Technical Notes:

- store desiccated at 4°C
- flash-freeze in experimental-sized aliquots upon reconstitution
- regard tubulin concentration, temperature, and guanosine addition when polymerizing
- do not place polymerized MTs on ice

Figure 1: Lyophilized Tubulin is >99% pure. Coomassie G250-stained protein gel of Lyophilized Tubulin separated by SDS-PAGE. The tubulin appears as a single species migrating at ~55 kDa. Molecular weight markers (kDa) and loaded protein quantities are indicated.

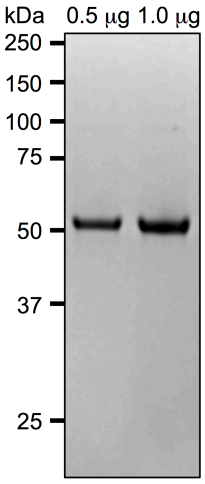
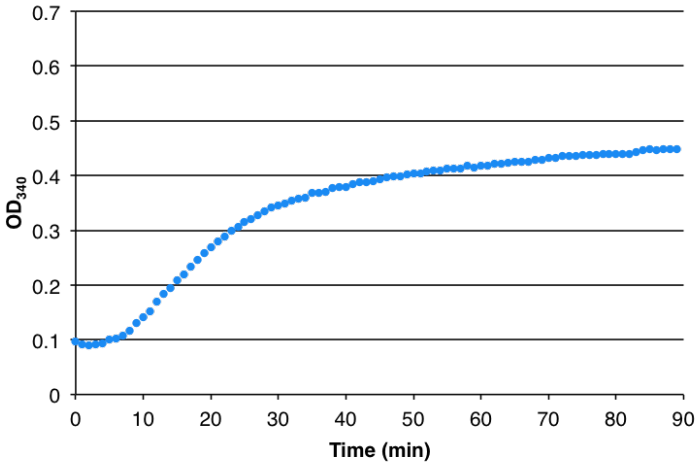


Figure 2: Lyophilized Tubulin is polymerization-competent. Optical Density (340 nm) of Lyophilized Tubulin at 5 mg/ml in Tubulin PEM Buffer (Cat. No. 032002; 80 mM PIPES, 1 mM EGTA, and 1 mM $MgCl_2$, pH 6.8) supplemented with 1 mM GTP, 1 mM DTT, and 5% Glycerol at 37°C. Distinct nucleation and polymerization phases are evident.



Comparison with Cycled Tubulin™ (Cat. No. 032005):

Specifications	Cycled Tubulin™	Lyophilized Tubulin
Cat. No.	032005	142001
Purity	>99%	>99%
Cycled	Yes	No
Storage Method	Cryopreserved	Lyophilized
Storage Buffer	PIPES	Phosphate
Price	< $150/mg	< $105/mg
Shipping Method	FedEx Overnight on Dry Ice	FedEx 2 Day Envelope

References:

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