DATASHEET: LYOPHILIZED TUBULIN (>99%)

Catalog Number: 142001
Source: Bovine Brain
Store Desiccated

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. When reconstituted with ultrapure water to 40 mg/ml, the buffer conditions are 10 mM Sodium Phosphate, 0.5 mM MgCl₂, 0.1 mM GTP, and 0.25 M Trehalose, pH 7.0. Note that 1 mg tubulin is supplied as 3 mg powder, and reconstitution/dilution should be based on tubulin concentration.

Storage and Handling:
Store Lyophilized Tubulin in a cool, dry environment. The product is stable under these conditions for 1 year. Reconstitute Lyophilized Tubulin by resuspending in ice-cold Tubulin PEM Buffer (Cat. No. 032002; 80 mM PIPES, 1 mM EGTA, and 1 mM MgCl₂, pH 6.8) to 40 mg/ml and incubating on ice for 15 minutes. Clarify the solution at 14,000 rpm for 1 minute at 4°C. 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. Note that 1 mg tubulin is supplied as 3 mg powder, and reconstitution/dilution should be based on tubulin concentration.

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
- kinase phosphorylation assays

Polymerization Protocol:
Dilute reconstituted Lyophilized Tubulin to 2 mg/ml with Tubulin PEM Buffer (Cat. No. 032002; 80 mM PIPES, 1 mM EGTA, and 1 mM MgCl₂, pH 6.8) 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 in a cool, dry environment
- 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 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 MgCl₂, pH 6.8) supplemented with 1 mM GTP and 20% glycerol at 37°C. Distinct nucleation and polymerization phases are evident.
Comparison with Cycled Tubulin (Cat. No. 032005):

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<tr>
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References: