

DATASHEET



CYCLED TUBULIN™

Catalog Number	Volume	Quantity
032005 - 1 mg	50 ul	1 mg
032005 - 20 mg	1000 ul	20 mg

STORE AT -80 ° C

Made in the USA

For research use only.

Shipping: shipped on dry ice

Storage Conditions: store at -80 ° C immediately

Form: clear aqueous solution

Source: bovine

Molecular Weight: ~110 kDa

Purity: >99% (SDS-PAGE)

Concentration: 20 mg/ml

Buffer Conditions: 80 mM PIPES, 1 mM EGTA, 1 mM MgCl₂ (pH 6.8)

Shelf Life: check product label for expiration date

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. Microtubules measure 25 nm in diameter and have a persistence length of ~2 μ m, incorporating ~1650 tubulin subunits per 1 μ m. Given the asymmetry of tubulin dimers, microtubules have inherent polarity with distinct "+" (β -tubulin exposed) and "-" (α -tubulin exposed) ends. Another critical feature of microtubules is their dynamic instability, a consequence of the GTPase activity of tubulin. This property confers force-generating capabilities to microtubules 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

Cycled Tubulin™ is isolated by cycling bovine brain homogenate through conditions that promote tubulin polymerization/depolymerization in high salt buffers by an adaptation of the method of Castoldi and Popov (2003). The resulting product is >99% pure (Figure 1) and polymerization competent (Figure 2). Cycled Tubulin™ is cryopreserved at 20 mg/ml in 1X Tubulin PEM Buffer (also known as BRB80; Cat. No. 032003; 80 mM PIPES, 1 mM EGTA, and 1 mM MgCl₂, pH 6.8).

Storage and Handling

Immediately transfer Cycled Tubulin™ to -80°C upon receipt. Thaw only when ready to use by placing briefly in a 37°C water bath followed by immediate placement on ice. Clarify the tubulin after thawing to remove any protein aggregates by centrifugation at 90k rpm (350k x g) for 5 minutes at 4°C. If desired, Cycled Tubulin™ can be aliquoted into smaller experimental batches, frozen in liquid Nitrogen, and stored at -80°C with minor loss of polymerization competency (Figure 2). Avoid repeated freeze-thaw cycles. View detailed storage and handling instructions at <https://puresoluble.com/storage-and-handling-cycled-tubulin/>.



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Activity and Applications

Cycled Tubulin™ will polymerize into microtubules when supplemented with guanosine-5'-triphosphate (GTP), warmed to 37 °C, and kept above its critical concentration. Polymerization activity is detectable in a variety of experimental systems including fluorescence microscopy assays, turbidity assays, and GTPase assays. Cycled Tubulin™ is suitable for use in a variety of cell-free experimental applications and can be combined with fluorescent or biotinylated proteins in generating microtubules *in vitro*. Visit www.PureSoluble.com/protocols for common microtubule polymerization protocols, including the generation of short, rigid microtubules stabilized by GMPCPP or long, flexible microtubules stabilized by taxol.

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

Figure 1: Cycled Tubulin™ is >99% pure.

Coomassie G250-stained protein gel of Cycled 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.

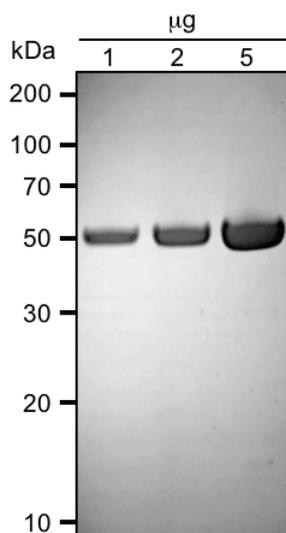
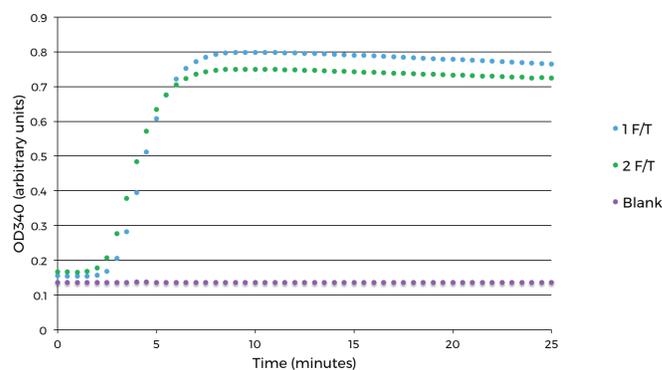


Figure 2: Cycled Tubulin™ is polymerization-competent.

Optical density (340 nm) of Cycled Tubulin™ that has undergone 1 (blue) or 2 (green) freeze/thaw cycles at 5 mg/ml in 1X Tubulin PEM Buffer (Cat. No. 032003; 80 mM PIPES, 1 mM EGTA, and 1 mM MgCl₂, pH 6.8) supplemented with 1 mM GTP and 20% glycerol and incubated at 37°C. Distinct nucleation and polymerization phases are evident.



References

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