**DATASHEET**

**CYCLED TUBULIN™**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Volume</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>032005 - 1 mg</td>
<td>50 ul</td>
<td>1 mg</td>
</tr>
<tr>
<td>032005 - 5 mg</td>
<td>250 ul</td>
<td>5 mg</td>
</tr>
<tr>
<td>032005 - 20 mg</td>
<td>1000 ul</td>
<td>20 mg</td>
</tr>
</tbody>
</table>

For research use only.

**Shipping:** shipped on dry ice  
**Purity:** >99% (SDS-PAGE)  
**Storage Conditions:** store at -80 °C immediately  
**Concentration:** 20 mg/ml  
**Form:** clear aqueous solution  
**Buffer Conditions:** 80 mM PIPES, 1 mM EGTA, 1 mM MgCl₂ (pH 6.8)  
**Source:** bovine  
**Molecular Weight:** ~110 kDa  
**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 mm, incorporating ~1650 tubulin subunits per 1 μm. 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 Tubulin PEM Buffer (also known as BRB80; Cat. No. 032002; 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 for 5 minutes at 4°C in an ultracentrifuge rotor (i.e. TLA 100). 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/.
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 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 and incubated at 37°C. Distinct nucleation and polymerization phases are evident.

References