DATASHEET

Labeled Tubulin-Alexa Fluor® 647

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Volume</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>064705 - 0.1 mg</td>
<td>5 ul</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>064705 - 0.5 mg</td>
<td>25 ul</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>064705 - 1.0 mg</td>
<td>50 ul</td>
<td>1.0 mg</td>
</tr>
</tbody>
</table>

For research use only.

Background
The microtubule network is a dynamic, force-generating cytoskeletal system essential for a number of basic cellular processes. Microtubules also serve as a track for kinesin and dynein motor proteins. As such, visualization of microtubules in real time, both in cells and in vitro, is critical in understanding cellular function and human disease. Tubulin, the basic component of microtubules, can be functionalized for visualization by covalent linkage with a fluorescent dye. Such modification must be performed in a way that maintains tubulin polymerization competency and functionality. The resulting labeled tubulin is useful in a number of applications ranging from live cell injection/imaging to in vitro nanoscale devices.

Material
Labeled Tubulin-Alexa Fluor® 647 is generated by reacting Alexa Fluor® 647 succinimidyl ester with Cycled Tubulin™ (Cat. No. 032005), thereby covalently linking the dye to random tubulin surface lysines. Cycled Tubulin™ is >99% pure and polymerization competent. These properties are maintained during the labeling process by reacting Cycled Tubulin™ with the dye in its polymerized form and subjecting it to a final polymerization/depolymerization cycle. The final product displays maximum absorption at 280 nm and 651 nm and a labeling stoichiometry ([dye]/[tubulin]) of ~0.5 as determined by spectroscopic analysis (Figure 1). Specific labeling stoichiometries are indicated on the product label. Labeled Tubulin-Alexa Fluor® 647 is commonly visualized with a Cy5 filter set with maximum excitation/emission wavelengths of 649/670 nm (Figure 2). The product is cryopreserved at 20 mg/ml in 50 mM K-Glutamate and 0.5 mM MgCl₂ (pH 7.0).

Storage and Handling
Immediately transfer Labeled Tubulin-Alexa Fluor® 647 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 labeled 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, Labeled Tubulin-Alexa Fluor® 647 can be aliquoted into smaller experimental batches, frozen in liquid Nitrogen, and stored at -80°C with minor loss of polymerization competency. Avoid repeated freeze-thaw cycles and protect from light. View detailed shipping and storage information on the product label.

For full product labeling, contact info@PureSoluble.com.

Made in the USA
storage and handling instructions at https://puresoluble.com/storage-and-handling-alexa-fluor-647/.

**Activity and Applications**
Labeled Tubulin-Alexa Fluor® 647 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. Labeled tubulin is suitable for use in a variety of fluorescent experimental applications including live cell injection, and can be combined with unlabeled tubulin in generating fluorescent microtubules in vitro. Cycled Tubulin™ (Cat. No. 032005) is highly recommended as the unlabeled tubulin source in order to minimize tubulin heterogeneity, as labeled tubulin products are derived directly from Cycled Tubulin™. 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.

- *in vitro* microurbule gliding assays
- single molecule kinesin and dynein motor assays
- speckle microscopy
- live cell injection
- *in vitro* nanoscale devices
- TIRF, STORM, SIM, STED, TPE, confocal, and widefield microscopy

**Figure 1:** Labeled Tubulin-Alexa Fluor® 647 displays maximum absorption at 280 nm and 651 nm.
Spectroscopic analysis reveals a peak at 280 nm and 651 nm, indicating absorption by the tubulin protein and Alexa Fluor® 647 dye, respectively.

**Figure 2:** Labeled Tubulin-Alexa Fluor® 647 is commonly visualized with a Cy5 filter set.
Taxol-stabilized microtubules at 5 mg/ml total tubulin protein with Labeled Tubulin-Alexa Fluor® 647 and Cycled Tubulin™ (Cat. No. 032005) at a 1:5 ratio.

**References**