DATASHEET

PurSolutions™

LABELED TUBULIN-ALEXA FLUOR® 647

Catalog Number	Volume	Quantity
064705	5 ul	0.1 mg

STORE AT -80˚C AND PROTECT FROM LIGHT

Made in the USA

For research use only.

Shipping: shipped on dry ice

Storage Conditions: store at -80˚C immediately

Form: blue aqueous solution

Source: bovine

Molecular Weight: ~110 kDa

Purity: >99% (SDS-PAGE)

Concentration: 20 mg/ml

Buffer Conditions: 50 mM K-Glutamate and 0.5 mM $MgCl_2$ (pH 7.0)

Maximum Excitation/Emission: λ=651/672 nm

Labeling Stoichiometry: ~0.5 (check product label)

Shelf Life: check product label for expiration date

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 >99% pure bovine protein, thereby covalently linking the dye to random tubulin surface lysines. Polymerization competency is maintained during the labeling process by reacting the dye with polymerized microtubules and subjecting the tubulin protein to a final polymerization/depolymerization cycle. The final labeled tubulin protein 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 (350k x g) for 5 minutes at 4°C. 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 storage and handling instructions at https://puresoluble.com/storage-and-handling-alexa-fluor-647/.

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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 (Cycled Tubulin™ highly recommended; Cat. No. 032005) in generating fluorescent 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.

- *in vitro* microubule 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.

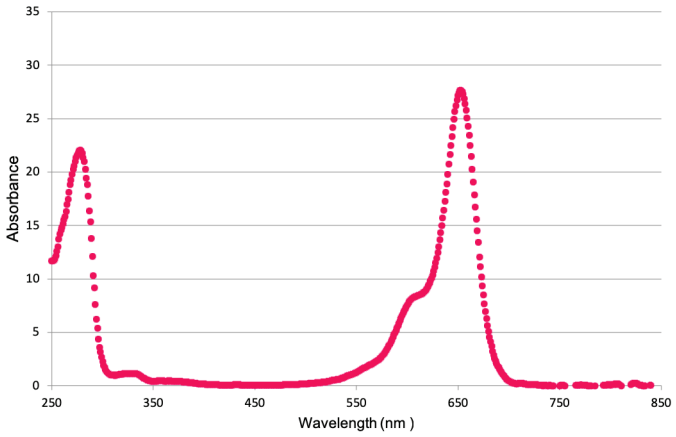
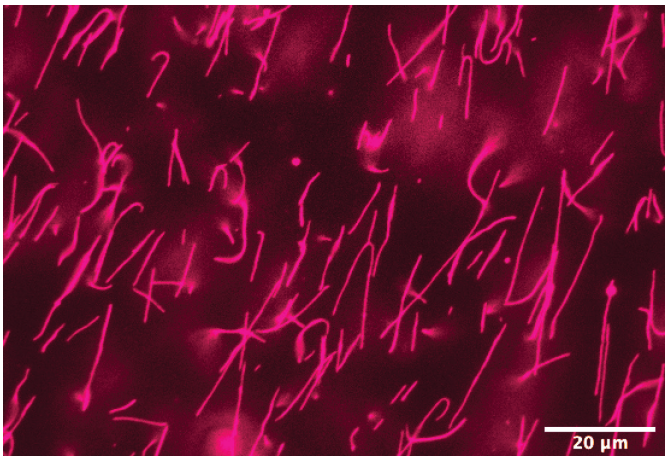


Figure 2: Labeled Tubulin-Alexa Fluor® 647 is commonly visualized with a Cy5 filter set.

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

1. Keith, C.H., Feramiscoa, J.R., Shelanski, M. Direct visualization of fluorescein labeled microtubules in vitro and in microinjected fibroblasts. J. Cell Biol. 88, 234–240 (1981).
2. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W.Y. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. J. Histochem. Cytochem. 47, 1179–1188 (1999).
3. Malcos, J.L. and Hancock, W.O. Engineering tubulin: microtubule functionalization approaches for nanoscale device applications. Appl. Microbiol. Biotechnol. 90(1), 1-10 (2011).