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PurSolutions LLC
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Charlottesville, VA 22901

DATASHEET: LABELED TUBULIN-ALEXA FLUOR® 594

Catalog Number: 059405
Source: Bovine Brain
Store at -80˚C

Background:

The microtubule (MT) network is a dynamic, force-generating cytoskeletal system essential for a number of basic cellular processes. MTs also serve as a track for kinesin and dynein motor proteins. As such, visualization of MTs in real time, both in cells and *in vitro*, is critical in understanding cellular function and human disease. Tubulin, the basic component of MTs, 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® 594 is generated by reacting Alexa Fluor® 594 NHS Ester (Thermo Fisher A20104) 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 absorbance at 590 nm and a labeling stoichiometry ([dye]/[tubulin]) of 0.5-1.5 as determined by spectroscopic analysis (Figure 1). Specific labeling stoichiometries are indicated on product labels. Labeled Tubulin-Alexa Fluor® 594 is commonly visualized with a Texas Red® filter set with maximum excitation/emission wavelengths of 590/617 nm. The product is cryopreserved at 20 mg/ml in 50 mM K-Glutamate and 0.5 mM $MgCl_2$ (pH=7.0).

Storage and Handling:

Immediately transfer Labeled Tubulin-Alexa Fluor® 594 to -80˚C upon receipt. The product is stable under these conditions for 1 year. Thaw only when ready to use by placing in a 37˚C water bath followed by immediate placement on ice. If desired, Labeled Tubulin-Alexa Fluor® 594 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.

Activity:

When supplemented with guanosine (GTP or GMPCPP) and warmed to 37˚C, Labeled Tubulin-Alexa Fluor® 594 will polymerize into MTs when above its critical concentration. It is recommended to combine Labeled Tubulin-Alexa Fluor® 594 with unlabeled tubulin at a ratio of 1:9 to a total tubulin concentration of 2 mg/ml. For unlabeled tubulin product options, refer to Cycled Tubulin™ (Cat. No. 032005) and Non-Cycled Tubulin (Cat. No. 142001).

Uses:
Labeled Tubulin-Alexa Fluor® 594 is supplied for use in fluorescent experimental systems including:

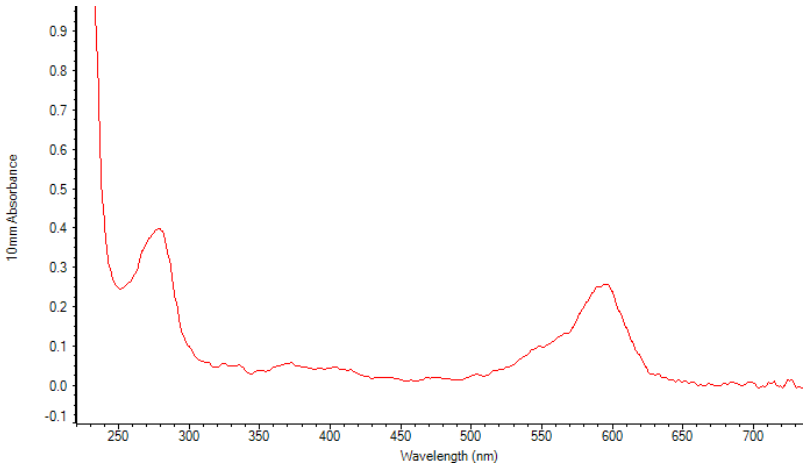
- TIRF, STORM, SIM, STED, TPE, confocal, and widefield microscopy applications
- *in vitro* MT gliding assays
- single molecule kinesin motor assays
- speckle microscopy
- *in vitro* nanoscale devices
- injection into live cells

Polymerization Protocol:
Dilute Labeled Tubulin-Alexa Fluor® 594 to 0.2 mg/ml with Tubulin PEM Buffer (Cat. No. 032002; 80 mM PIPES, 1 mM EGTA, and 1 mM $MgCl_2$, pH 6.8) and supplement with 1.8 mg/ml Cycled Tubulin™ (Cat. No. 032005), 1 mM DTT, and 1 mM 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. Protect polymerized MTs from light.

Technical Notes:

- store at -80°C
- protect from light
- avoid repeated freeze-thaw cycles, refreeze in liquid Nitrogen if required
- thaw only when ready to use at 37° C followed by immediate placement on ice
- regard tubulin concentration, temperature, and guanosine addition when polymerizing
- do not place polymerized MTs on ice

Figure 1: Labeled Tubulin-Alexa Fluor® 594 displays maximum absorption at 280 nm and 590 nm. Spectroscopic analysis reveals a peak at 280 nm and 590 nm, indicating absorption by tubulin and Alexa Fluor® 594, respectively. Spectroscopic analysis further reveals a labeling stoichiometry of 0.5-1.5. Specific labeling stoichiometries are indicated on the product label.



Comparison with other labeled tubulins:

Specifications	Alexa Fluor® 488	Alexa Fluor® 594	Alexa Fluor® 647
Cat. No.	048805	059405	064705
Purity	>99%	>99%	>99%
Cycled	Yes	Yes	Yes
Ex/Em	494/517 nm	590/617 nm	651/672 nm
Storage Method	Cryopreserved	Cryopreserved	Cryopreserved
Shipping Method	FedEx Overnight on Dry Ice	FedEx Overnight on Dry Ice	FedEx Overnight on Dry Ice

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).