

DATASHEET



LABELED TUBULIN-BIOTIN-XX

Catalog Number	Volume	Quantity
033305 - 0.1 mg	5 ul	0.1 mg
033305 - 0.5 mg	25 ul	0.5 mg
033305 - 1.0 mg	50 ul	1.0 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: 50 mM K-Glutamate and 0.5 mM MgCl₂ (pH 7.0)

Labeling Stoichiometry: ~4.0 (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 force-generating kinesin and dynein motor proteins. As such, immobilization of microtubules onto solid surfaces such as glass coverslips and beads allows for the biophysical study of these molecular machines. Tubulin, the basic component of microtubules, can be functionalized by covalent linkage with Biotin-XX, a long-chain biotin derivative. Such modification must be performed in a way that maintains tubulin polymerization competency and functionality. The resulting biotinylated tubulin is useful in a number of applications ranging from optical traps to tubulin affinity columns.

Material

Labeled Tubulin-Biotin-XX is generated by reacting Biotin-XX succinimidyl ester with Cycled Tubulin™ (Cat. No. 032005), thereby covalently linking the biomolecule 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 reactive biotin in its polymerized form and subjecting it to a final polymerization/depolymerization cycle. The final product demonstrates an affinity toward streptavidin-coated beads (Figure 1) and streptavidin-coated coverslips (Figure 2). Specific labeling stoichiometries are indicated on the product label. 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-Biotin-XX 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 biotinylated 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-Biotin-XX 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. View detailed storage and handling instructions at <https://puresoluble.com/storage-and-handling-biotin-xx/>.



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Activity and Applications

Labeled Tubulin-Biotin-XX 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. Biotinylated tubulin is suitable for use in a variety of experimental applications and can be combined with other labeled or unlabeled tubulin proteins in generating 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.

- optical traps and molecular tweezers
- single molecule kinesin and dynein motor assays
- tubulin affinity columns
- *in vitro* nanoscale devices

Figure 1: Labeled Tubulin-Biotin-XX binds to streptavidin-coated beads.

Coomassie G250-stained protein gel of unbound and bound fractions of tubulin protein samples incubated with Dynabeads® M-280 Streptavidin (Thermo Fisher 11205D). Cycled Tubulin™ serves as the non-biotinylated negative control. Labeled Tubulin-Biotin-XX fractionates with the streptavidin-coated beads.

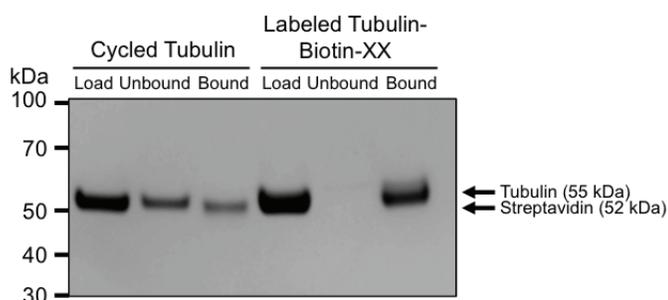
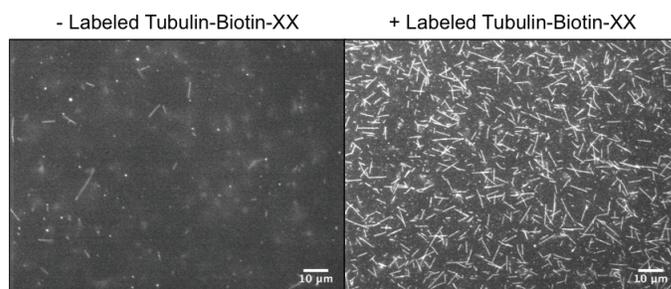


Figure 2: Labeled Tubulin-Biotin-XX binds to streptavidin-coated coverslips.

Fluorescent images of microtubules incubated in streptavidin-coated flow cells. Microtubules were polymerized with 1.8 mg/ml Cycled Tubulin™ and 0.2 mg/ml Labeled Tubulin-Alexa Fluor® 647 +/- 0.05 mg/ml Labeled Tubulin-Biotin-XX. Microtubules polymerized with Labeled Tubulin-Biotin-XX are bound to the flow-cell surface.



References

1. Malcos, J.L. and Hancock, W.O. Engineering tubulin: microtubule functionalization approaches for nanoscale device applications. *Appl. Microbiol. Biotechnol.* 90(1), 1-10 (2011).

