V. 1.1

Biotin—Labeled HeLa Tubulin

Cat. # H003

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Uses

1. Screening for cancer tubulin specific ligands.
2. Using SPA technology (Amersham Inc.) for drug screening.
3. Microinjection and tubulin distribution in living cells.
4. Immobilizing tubulin onto a solid surface via streptavidin.

Material

HeLa cell tubulin has been modified so that random surface lysines contain a covalently linked, long-chain biotin derivative. An activated ester of the biotin derivative is used in the labeling procedure. A long-chain biotin derivative was selected for this procedure because it allows the biotin molecules to be spaced far enough away from the tubulin protein so as not to interfere with its activity, e.g., ligand binding to SPA and other streptavidin-based reagents. Labeling stoichiometry was determined to be approximately one biotin per tubulin heterodimer (see Figure 1). Biotin-labeled tubulin is supplied as a lyophilized powder. The white powder is present usually at the bottom of the tube, but occasionally it may move to another location, so be sure to perform a short microfuge to collect the powder in the bottom of the tube prior to use. There is 40 ug of H003 minimum quantity per tube.

Figure 1 - Detection of biotin HeLa tubulin and its biological activity

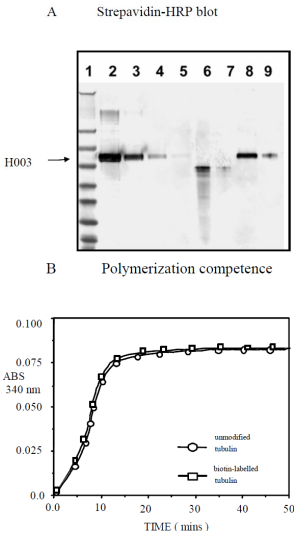

Figure 1: A = Lane 1 – MWM Invitrogen, Lane 2 – 1000ng of H003, Lane 3 – 100ng of H003, Lane 4 – 10ng of H003, Lane 5 – 1ng of H003, Lane 6 – 100ng of biotin actin (Cat.#AB07, Lane 7 - 10ng of AB07, Lane 8 – 100ng of biotin tubulin (Cat.# T333), Lane 9 – 10ng of biotin tubulin (Cat.# TL238). Probed with 1/1000 dilution of streptavidin/alkaline phosphatase conjugate (Sigma Co.) and developed with BCIP/NCT solution from Pierce Co. **B** = Biological activity of biotin HeLa tubulin; as determined by Microtubule assembly characteristics. Method: 250ug of biotin HeLa tubulin were reconstituted to 2mg/ml in G-PEM buffer pH6.9 plus 20% v/v glycerol and incubated at 35°C in a cuvette. The optical density at 340 nm was taken at time intervals. A value of 0.075 OD unit indicates that >90% of the tubulin has polymerized.

Reconstitution Instructions

For 5.0 mg/ml biotin tubulin, pipette 8.0 ul (40 ug vial) of ice cold G-PEM buffer plus 5% glycerol into the bottom of the tube (G-PEM: 80 mM Piperazine-N,N'-bis[2-ethanesulfonic acid sequisodium salt; 2 mM magnesium chloride; 0.5 mM ethylene glycol-bis(b-amino-ethyl ether) N,N,N,N'-tetra-acetic acid; 1 mM guanosine 5'-triphosphate, pH 6.9). Pipette up and down for approximately 5 seconds to mix and dissolve the protein, then place on ice until ready to use. Tubulin at 5.0 mg/ml can be aliquoted into smaller sizes and snap-frozen in liquid nitrogen. These frozen stocks are stable for 6 months at -70°C. Working stocks of the biotin tubulin can be made by further diluting the protein in G-PEM plus 5% glycerol.

Biotin tubulin may also be diluted with unlabeled tubulin to create a biotin tubulin substrate with a stoichiometry of less than one biotin per tubulin heterodimer. Unlabeled HeLa tubulin (cat # H001) can be purchased from Cytoskeleton Inc.

Polymerization of H003

Biotin HeLa cell tubulin has polymerization properties that are indistinguishable from those of unlabeled tubulin (see Figure 1B). Polymerizaton conditions are given in the legend to Figure 1B.

Working with heterodimeric (unpolymerized) H001

If tubulin is diluted in G-PEM to below 1 mg/ml and kept at between 4-24°C it will be maintained as tubulin heterodimers (i.e., monomers). It should be noted that tubulin is a labile protein and it should be used or snap-frozen as soon as possible after reconstitution.

Purity

Purity is determined by scanning densitometry of proteins on SDS-PAGE gels. Samples are ≥90% pure.

Storage

Store at -70°C in sealed bag or a reconstituted frozen liquid. Stable for 6 months. Do not store tube with lyophilized product in a non-sealed bag.

Product Citations/Related Products

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