V. 1.0

Biotin Labeled Muscle Actin

Cat. # AB07

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

Material
Rabbit skeletal muscle actin (Cat.# AKL99) has been modified to contain covalently linked biotin at random surface lysine residues. An activated ester of biotin is used to label the protein. The labeling stoichiometry has been determined to be approximately 1 biotin per actin monomer. Biotinylated actin has an approximate molecular weight of 43 kDa. AB07 (20 µg of protein) is supplied as a lyophilized powder.

Storage and Reconstitution
The lyophilized protein when stored desiccated to < 10% humidity at 4°C is stable for 6 months. The protein should be reconstituted to 10 mg/ml with 2 µl of distilled water. The protein will then be in the following buffer: 5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, 5% (w/v) sucrose, and 1% (w/v) dextran. The concentrated protein should then be aliquoted and snap frozen in liquid nitrogen. The protein is stable for 6 months if stored at -70°C. For working concentrations, further dilution of the protein should be made with General Actin Buffer (Cat.# BSA01) supplemented with 0.2 mM ATP (Cat.# BSA04) and 0.5 mM DTT.

Purity
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gel. Biotinylated actin was found to be >99% pure. (see Figure 1.)

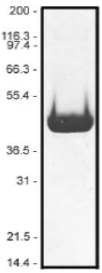

Figure 1. Biotinylated Actin Protein Purity Determination. A 10 µg sample of biotinylated actin (molecular weight approx. 43 kDa) was separated by electrophoresis in a 12% SDS-PAGE system. The protein was stained with Coomassie Blue. Protein quantitation was determined with the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Sensitivity of Biotin Detection
To determine the efficiency of biotin labeling, nanogram amounts of biotinylated actin were separated by electrophoresis and electroblotted onto a Nitrocellulose membrane. The blot was then probed with streptavidin linked alkaline phosphatase. The biotin label on actin was detected down to the 1 ng level of protein (see Figure 2). No free label is apparent in the final product.

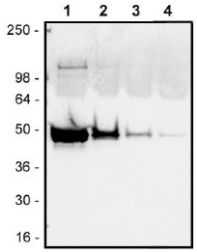

Figure 2. Detection of 1 ng of biotinylated actin. Serial dilutions of biotinylated actin were separated by electrophoresis on a 12% polyacrylamide gel and blotted onto Nitrocellulose. The membrane was then probed with streptavidin alkaline phosphatase (Sigma) and detected with the 1-Step NBT/BCIP reagent (Pierce). Lane 1. 1000 ng, Lane 2. 100 ng, Lane 3. 10 ng, and Lane 4. 1 ng of biotinylated actin. See Blue molecular weight markers are from Invitrogen.

Biological Activity Assay
The biological activity of biotinylated actin can be determined from its ability to efficiently polymerize into filaments *in vitro* and separate from unpolymerized components in a spin down assay. Stringent quality control ensures that >85% of the biotinylated actin can polymerized in this assay. This is comparable to the polymerization capacity of unmodified actin (Cat. # AKL99). The assay is carried out as outlined below.

Reagents
1. Biotin labeled actin (Cat. # AB07)
2. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$) (Cat. # BSA01)
3. Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP) (Cat. # BSA02)
4. ATP 100 mM solution (Cat. # BSA04)
5. Precision Red Protein Assay Reagent (Cat. # ADV02)

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Equipment

- Microfuge at 4°C
- Beckman airfuge or ultracentrifuge (capable of 100,000 x g, 200 µl volume)
- Ultracentrifuge tubes (Beckman Cat. # 344718)
- Spectrophotometer

Method

1. Resuspend biotin labeled actin to 0.4 mg/ml in General Actin Buffer supplemented with ATP to a final concentration of 0.2 mM.
2. Incubate on ice for 60 min to depolymerize actin oligomers that form during storage.
3. Centrifuge the protein in a 4°C microfuge at 14k rpm for 15 min.
4. Transfer the supernatant to a new microfuge tube and determine the total protein concentration with the Precision Red Protein Assay Reagent.
5. Aliquot 200 µl of the actin solution to an ultracentrifuge tube.
6. Add 20 µl (1/10th the volume) of Polymerization Buffer to the airfuge tube and mix well.
7. Incubate at room temperature for 1 h.
8. Centrifuge the tubes at 100,000 x g for 1 h. to pellet the polymerized actin.
9. Remove the top 90% of the supernatant to a clean microfuge tube.
10. Determine the concentration of the protein in the supernatant (unpolymerized monomer actin) with the Precision Red Protein Assay Reagent. This protein concentration is used to determine the efficiency with which the biotinylated actin polymerized and pelleted during centrifugation.

Product Uses

- Microinjection into muscle cell followed by electron microscopy of streptavidin conjugated gold particles to determine the cellular localization of the modified actin.
- *In vitro* motility experiments using biotinylated seeds of F-actin bound to fluorescent beads for laser tweezer manipulation.
- Produce affinity columns using biotinylated F-actin or G-actin to extract and purify novel proteins.
- Purify proteins or develop bioassays using biotin F-actin or G-actin with streptavidin-magnetic beads.

Product Citations/Related Products

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