V. 2.1

Actin: Biotin Labeled
(Rabbit skeletal muscle, >99% pure)
Cat. # AB07-A
Lot # 053 Amount: 5 x 20 µg
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 and is supplied as a lyophilized powder (white).

Applications

Application	Reference
Actin organization & its impact on ABP function and motion	1, 2
Modeling *in vitro* bio membranes	3, 4
Molecular mechanisms underlying skeletal mediated force/stress	5, 6
Actin & microtubule coupling, mechanical properties, & dynamics	7, 8
Motor communication & function: motility assays, optical tweezers & optical traps	9, 10, 11, 12
Study actin binding proteins	13, 14
Applications in functional nanodevices	15,16

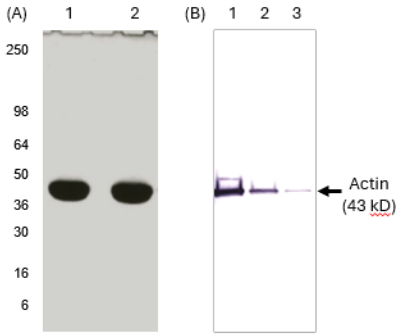
Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The lyophilized protein is stable for 6 months when stored desiccated to <10% humidity at 4°C. The protein should be reconstituted to 10 mg/ml with 2 µl of nanopure water; it 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 can then be snap frozen in liquid nitrogen and stored at -70°C where it is stable for 6 months. 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. Biotinylated muscle actin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

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 1A)

Figure 1. Protein Purity and Detection of Biotinylated Actin



Legend Fig 1: Fig 1A: A 20 µg sample of biotinylated actin (Lanes 1 & 2) 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.
Fig 1B: Serial dilutions of biotinylated actin were separated by electrophoresis on a 12% polyacrylamide gel and blotted onto PVDF. The membrane was then probed with streptavidin alkaline phosphatase and detected with the 1-Step NBT/BCIP reagent (Pierce). Lane 1. 100 ng, Lane 2. 10 ng, Lane 3. 1 ng of biotinylated actin.

Sensitivity of Biotin Detection

To determine the efficiency of biotin labeling, nanogram amounts of biotinylated actin were separated by electrophoresis and electroblotted onto a PVDF 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 1B). No free label is apparent in the final product.

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Quality Control: Biological Activity Assay

The biological activity of biotinylated actin has been determined by its ability to efficiently polymerize into filaments *in vitro* and separate from unpolymerized components in a spin down assay. Stringent quality control ensures that >90% 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.

***In vitro* polymerization of biotin actin to create biotinylated actin filaments**

1. Resuspend biotinylated actin to 0.4 mg/ml with General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$) (Cat. # BSA01) supplemented with 0.2 mM ATP and 1 mM DTT.

2. Add 1/10th the volume of 10X Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP) (Cat. # BSA02) supplemented with 1 mM DTT and incubate at room temperature for 1 h to form biotinylated actin filaments. Filaments are stable for several days at room temperature or 4°C.

3. Filaments can be further diluted in 1X Polymerization Buffer plus 70 nM phalloidin for downstream applications. Phalloidin is an actin filament stabilizer, care should be taken when handling phalloidin as it is a toxic compound.

Advice for Working with Muscle Actin

1. Monomer actin is unstable in the absence of ATP (0.2 mM), a divalent cation (0.2 mM $CaCl_2$ or 10 µM $MgCl_2$) and dithiothreitol (1 mM)
2. Monomer actin will polymerize at >20 mM K+, Na+, and in >0.2 mM Mg^{2+}.
3. Monomer actin will not polymerize at <2 mM K+, Na+, or in <0.05 mM Mg^{2+}.
4. Monomer actin is unstable below pH 6.5, or above pH 8.5.
5. Snap freeze actin in liquid nitrogen at 10 mg/ml to maintain high biological activity.

Application References

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