



Muscle Actin > 99% pure
Rabbit Skeletal Muscle
Cat. # AKL99-A
Lot # 092
Store at 4°C (desiccated) or at -70°C

Material

Actin protein has been purified from rabbit skeletal muscle by the method of Pardee and Spudich (1). AKL99 actin is greater than 99% pure. Muscle actin has an approximate molecular weight of 43 kDa. AKL99 (250 ug of protein per tube) is supplied as a lyophilized powder.

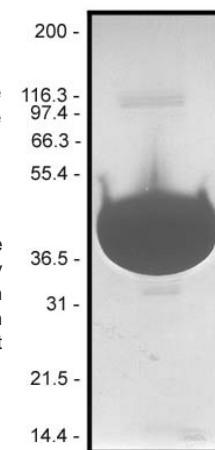
Storage and Reconstitution

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 25 ul of distilled water. The protein will then be in the following buffer: 5 mM Tris-HCl pH 8.0, 0.2 mM CaCl₂, 0.2 mM ATP, 5% (w/v) sucrose, and 1% (w/v) dextran. The concentrated protein should then be aliquoted into experiment sized aliquots and snap frozen in liquid nitrogen and stored at -70°C. 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. 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. Muscle actin was found to be >99% pure (see Figure 1).

Figure 1. Muscle Actin Protein Purity Determination. A 100 ug sample of muscle 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.



Biological Activity Assay

The biological activity of muscle actin can be 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 muscle actin can be polymerized in this assay.

Reagents

- 1) Muscle Actin (Cat. # AKL99)
- 2) General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM CaCl₂) (Cat. # BSA01)
- 3) Polymerization Buffer (500 mM KCl, 20 mM MgCl₂, 10 mM ATP) (Cat. # BSA02)
- 4) ATP, 100 mM solution (Cat. # BSA04)
- 5) 1 M DTT (dithiothreitol)
- 6) Precision Red Protein Assay Reagent (Cat. # ADV02)

Equipment

- 1) Microfuge at 4°C
- 2) Beckman Airfuge and Ultra-Clear™ centrifuge tubes (Cat. # 344718), Beckman ultracentrifuge and SW 55 Ti rotor with Ultra-Clear™ centrifuge tubes (Cat. # 344718) and adapters (Cat. # 356860), or other ultracentrifuge capable of centrifuging 200 µl at 100,000 x g.
- 3) Spectrophotometer capable of measuring absorbance at 600 nm.

Method

- 1) Resuspend muscle actin to 0.4 mg/ml in General Actin Buffer supplemented with 0.2 mM ATP and 0.5 mM DTT.
- 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 each 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 of each tube 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 actin polymerized and pelleted during centrifugation.

% polymerization for Lot 092 = 94%

Advice for Working with Muscle Actin

- 1) Monomer actin is unstable in the absence of ATP, a divalent cation and dithiothreitol (DTT)
- 2) Monomer actin will polymerize at >2 mM K⁺, Na⁺, and in > 0.05 mM Mg²⁺.
- 3) Monomer actin is unstable below pH 6.5, or above pH 8.5.
- 4) Polymerized actin is more resilient to adverse conditions than monomeric actin. Therefore, actin is preferably stored in the polymerized form at 4°C for several weeks. If filaments are to be stored for longer than 24 h, addition of an antibacterial agent such as 0.05% sodium azide or 100 µg/ml ampicillin and 10 µg/ml chloramphenicol is recommended.
- 5) Snap freeze actin in liquid nitrogen at 10 mg/ml to maintain high biological activity for 6 months.

Product Uses

- * Identification and characterization of muscle actin binding proteins
- * In Vitro actin polymerization studies
- * Antibody standard for Western blot analysis

References

- (1). Pardee J.D., and Spudich, J.A. 1982. *Methods in Cell Biol.* 24:271-288.

Related Products and Uses

Cytoskeleton Inc. offers the widest range of actin products currently available. We specialize in producing highly purified proteins with high biological activity. These include the actin Biochem Kits, actin associated proteins, and other reagents for actin based assays. Included in the actin product line are:

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| * Actin Binding Protein Assay Kit | Cat. # BK001 |
| * Actin Polymerization Biochem Kit | Cat. # BK003 |
| * In Vivo G-Actin / F-Actin Assay Kit | Cat. # BK037 |
| * Actin protein (rabbit skeletal muscle, >95% pure) | Cat. # AKL95 |
| * Actin protein (human platelet, non-muscle, >95% pure) | Cat. # APHL95 |
| * Actin protein (human platelet, non-muscle, >99% pure) | Cat. # APHL99 |
| * Actin protein (bovine cardiac muscle, >99% pure) | Cat. # AD99 |
| * Biotin actin protein (human platelet, non-muscle) | Cat. # APHB |
| * Biotin actin protein (rabbit skeletal muscle, >99% pure) | Cat. # AB07 |
| * Pyrene actin protein (rabbit skeletal muscle, >99% pure) | Cat. # AP05 |
| * Rhodamine actin protein (rabbit skeletal muscle) | Cat. # AR05 |
| * Rhodamine actin protein (human platelet, non-muscle) | Cat. # APHR |
| * Rhodamine phalloidin | Cat. # PHDR |