

Cardiac Actin >99% pure
Bovine Heart Muscle
Cat. # AD99

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

Material

Actin has been purified from bovine heart muscle by the method of Pardee and Spudich (1). Bovine cardiac actin is composed of 84% α_{cardiac} and 16% α_{skeletal} actin isoforms (2) and has an approximate molecular weight of 43 kDa. Bovine cardiac actin is greater than 99% pure actin and is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. 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 100 μ l 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 into experiment sized amounts, 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, see reagent list) 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. Cardiac actin was found to be >99% pure (see Figure 1).

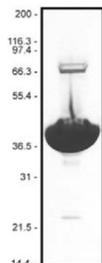


Figure 1. Cardiac Actin Protein Purity Determination. A 100 μ g sample of bovine cardiac actin (molecular weight approx. 43 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined with the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of cardiac 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 polymerized in this assay.

Reagents

1. Bovine Cardiac Actin (Cat. # AD99)
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. 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 μ l at 100,000 x g.
3. Spectrophotometer capable of measuring absorbance at 600 nm.

Method

1. Dilute the cardiac 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 1 h 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 μ l of the actin solution to an ultracentrifuge tube.
6. Add 20 μ l ($1/10^{\text{th}}$ 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.

Advice for Working with Cardiac 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 Mg^{2+} .
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 polymeric form at $4^{\circ}C$ 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 $\mu g/ml$ ampicillin and 10 $\mu g/ml$ 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 cardiac 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.
2. Vandekerckhove, J., et al. 1986. *JBC.* 261:1838-1843.

Product Citations/Related Products

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