Non-muscle Actin >99% pure (human platelet)
Cat. # APHL99

V 2.1

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

Material

Non-muscle actin has been purified from human platelets. Each unit of platelets used in the preparation of non-muscle actin has been found to be non-reactive by an FDA approved test for HBsAg, HBcAb, HIV-1/2 ab, HIV-1 RNA, HTLV I/II ab, HCV ab, HCV RNA, and syphilis. Each unit of platelets has been ALT tested with results less than an established cutoff. The isotype composition of non-muscle actin is 85% β-actin and 15% γ-actin. Non-muscle actin has an approximate molecular weight of 43 kDa. APHL99 is provided as a lyophilized white powder.

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 deionized water. Reconstitution to 10 mg/ml is best accomplished by occasional pipetting on ice or at 4°C for 10-15 minutes, and will result in an opaque solution. Do not heat the actin during reconstitution. After reconstitution the actin will 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) supplemented with 0.2 mM ATP (Cat. # BSA04) and 0.5 mM DTT. 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. Non -muscle actin was found to be >99% pure (see Figure 1).

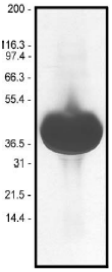

Figure 1. Non-muscle Actin Protein Purity Determination. A 100 µg sample of non-muscle actin (molecular weight approx. 43 kDa) was separated by electrophoresis in a 12% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent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

Biological Activity Assay

The biological activity of non-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 >85% of the non-muscle actin can polymerize in this assay.

Reagents

1) Non-muscle actin (Cat. # APHL99)
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. # BSA01
4) 100 mM ATP solution (Cat. # BSA04)
5) 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 the non-muscle actin to 0.4 mg/ml in General Actin Buffer supplemented with 0.2 mM ATP.
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 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.

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Advice for Working with Non-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 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 polymerized form at 4°C for two 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.

Product Uses

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

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.