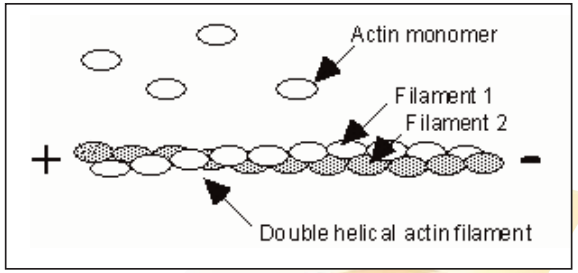
G-actin polymerizes to form F-actin

Globular-actin (G-actin) readily polymerizes under physiological conditions to form Filamentous-actin (F-actin) with the concomitant hydrolysis of ATP. F-actin is a double-helical filament as shown below:

Figure 2. Double-helical structure of actin filaments



Actin can polymerize from both ends *in vitro*, however, the rate of polymerization is not equal. This results in an intrinsic polarity in the actin filament. It has therefore become the convention to term the rapidly polymerizing end the plus-end (see Figure 2) or barbed-end while the slow growing end is called the minus-end or pointed-end.

The propensity of actin to polymerize is dependent upon the affinity of actin monomers for filament ends. Thus, there is an actin monomer concentration below which actin will not polymerize; this value has been termed the Critical Concentration (CC). At monomer concentrations above the CC, the actin will polymerize until the free monomer concentration is equal to the CC. When one is working with actin in vitro the extent of actin polymerization depends upon the conditions used. For example, at 4°C muscle actin has a CC of 0.03 mg/ml in the presence of Mg^{2+} (2 mM) and KCl (50 mM), but when these ions are absent, the CC is approximately 3.0 mg/ml. Thus, by altering the ionic type and strength one can alter the amount of polymer formed. For more information see reference 2.

Conditions in which F-actin is stable

G-actin is stable for two days at 4°C. F-actin is stable can be stored at 4°C for one week. F-actin can be transferred to a variety of buffers (e.g. HEPES, phosphate, etc) without detrimental effects. G-Actin requires a divalent cation, pH 6.5 - 8.0 and ATP for stability. F-actin also requires Mg^{2+} for stability. If you have any further questions about purified actins please call our technical assistance at 303-322-2254.

Pre-formed Actin Filaments

Rabbit Skeletal Muscle
Cat. # AKF99
Lot # 011 Amount: 1 x 1 mg
Store at 4ºC (desiccated).

Material

Actin protein has been purified from rabbit skeletal muscle by the method of Pardee and Spudich (1) to greater than 99% purity. Skeletal muscle actin is composed of 99.5% alpha-actin and has an approximate molecular weight of 43 kDa. Actin was polymerized at 0.4 mg/ml (10 µM) in 5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1.0 mM DTT and 2.0 mM $MgCl_2$ to form 5 to 10 µm long filaments. Filament preparations are stabilized with 5% (w/v) sucrose and then lyophilized. The filaments are supplied as a white lyophilized powder.

Biological Activity

If a solution of AKF99 is sedimented at 100,000 xg for 1 h, greater than 85% of the protein can be found in the pellet (F-actin) compared to the supernatant (G-actin). Lot 011 - PASS.
In an F-actin activated myosin ATPase assay there is greater than a ten fold increase in ATPase activity with AKF99 compared to a reaction without filaments. Lot 011 - PASS.

Storage and Reconstitution

The lyophilized protein is stable for 6 months when stored desiccated to <10% humidity at 4˚C. The protein should be reconstituted to 0.4 mg/ml with 2.4 ml of Milli-Q water at RT. 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, 2mM $MgCl_2$ and 5% (w/v) sucrose. Filaments can be stored at 4˚C for up to 1 week, or aliquoted into <100 µl samples and snap frozen in liquid nitrogen and stored at -70˚C. The protein is stable for 6 months if stored at -70˚C. Defrost rapidly in a 37˚C waterbath, avoid more than one freeze-thaw cycle. Further dilution of the protein should be made with 5 mM Tris-HCl pH 8.0, 0.2 mM ATP and 2 mM $MgCl_2$ or 0.2 mM $CaCl_2$.

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). Contaminants are actin doublets and α-actinin.

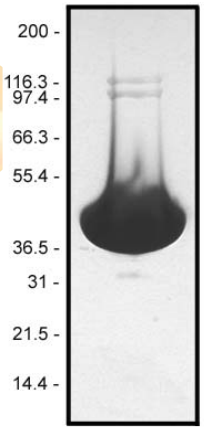


Figure 1. ***Actin Protein Purity Determination.*** A 100 µg sample of muscle actin (molecular weight approx. 43 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined with the Precision Red Protein Assay Reagent (Cat. # ADV02).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

Biological Activity Assay

The biological activity of F-actin can be determined by its sedimentation character and its ability to activate myosin ATPase. Stringent quality control ensures that >85% of the actin is present as filaments and that the myosin ATPase is at least ten fold stimulated over myosin or actin alone background control values.

Reagents

1) Pre-formed Filaments of Rabbit Muscle Actin (Cat. # AKF99)
2) Rabbit skeletal muscle myosin (Cat. # MY02)
3) Reaction Buffer (ice cold 20 mM Tris-HCl pH 7.5, 15 mM KCl, 6 mM $MgCl_2$ and 2 mM EGTA)
4) ATP, 100 mM solution (Cat. # BSA04), dilute 10ul into 1 ml of ice cold Milli-Q water for 1mM.
5) Precision Red Protein Assay Reagent (Cat. # ADV02)

Equipment

1) Microfuge at 4˚C
2) Beckman Airfuge and Ultra-Clear™ centrifuge tubes (Cat. # 344718) or Beckman ultracen trifuge 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 and 650 nm.

Method

1) Resuspend 1 mg AKF99 with 2.4 ml of Milli-Q water at room temperature (RT).
2) Incubate at RT for 10 min to allow filaments to dissociate from each other.
3) Resuspend myosin to 2 mg/ml with 500 µl of ice cold Milli-Q water. Incubate on ice for 5 min to completely solubilize the protein.
4) Mix 30 µl reactions in a well of a 1/2 area 96-well plate (Corning Cat. # 3696 or 3697) containing:
   8 µl of Reaction Buffer
   12 µl of Pre-formed Actin Filaments or Reaction Buffer plus 0.2mM ATP as a control
   2 µl of Myosin solution or Milli-Q water as a control
   The order of addition to the reaction is very important, i.e. add myosin last.
5) Initiate the reactions by adding 8 µl of 1mM ATP and shake the plate for 5 s.
6) Incubate at 37°C for 10min in a waterbath.
7) Pipette 150µl of CytoPhos™ reagent (Cat.# BK054) into each well.
8) Incubate for exactly 5 or 10min at RT then read at 650 nm. (5 min may give less background)
9) Compare the readings at OD650 with a phosphate standard curve (0.5mM K_2HPO_4 0, 2, 5, 10 and 30 µl) to calculate the specific activity in nmoles/min/mg myosin.
10) Note: Protein concentrations were determined by using the Precision Red Advanced Protein Assay reagent (Cat. ADV02).

Advice for Working with Pre-formed Actin Filaments

1) Pre-formed Actin Filaments are unstable in the absence of ATP and $MgCl_2$.
2) Pre-formed Actin Filaments are unstable below pH 6.5, or above pH 8.5.
3) Pre-formed Actin Filaments can be stored up to one week at 4°C, addition of antibacterial agents such as 100 µg/ml ampicillin and 10 µg/ml chloramphenicol is recommended.
4) Pre-formed Actin Filaments should not be frozen more than once in liquid nitrogen or in volumes greater than 100 µl.

Product Uses

* Myosin ATPase assays
* F-actin binding assays
* Iodinated for blot overlay experiments

References

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

2) Gordon D.J. et al. 1977. *JBC*, **252**, 8300-8309.

Related Products

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:

* 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 (bovine cardiac muscle, >99% pure)	Cat. # AD99
* Actin protein (rabbit skeletal muscle, >95% pure)	Cat. # AKL95
* Actin protein (rabbit skeletal muscle, >99% pure)	Cat. # AKL99
* Actin protein (human platelet, non-muscle, >95% pure)	Cat. # APHL95
* Actin protein (human platelet, non-muscle, >99% pure)	Cat. # APHL99
* Actin protein (chicken gizzard smooth muscle, >99% pure)	Cat. # AS99
* 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. # PHDR1