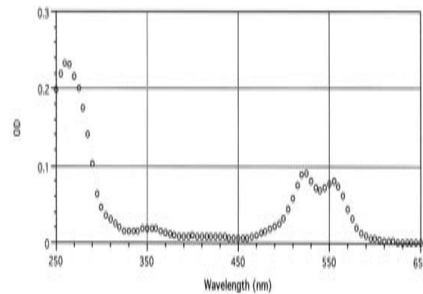


Figure 3. Absorption scan of rhodamine actin in solution. Rhodamine muscle actin was diluted with Milli-Q water in a microtiter plate well according to the method and its absorbance spectrum scanned between 250 and 650 nm. Labeling stoichiometry was calculated to be 0.5 dyes per actin monomer.



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 >20 mM K⁺, Na⁺, and in >0.2 mM Mg²⁺.
- 3) Monomer actin will not polymerize at <2 mM K⁺, Na⁺, or in <0.05 mM Mg²⁺.
- 4) Monomer actin is unstable below pH 6.5, or above pH 8.5.
- 5) 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.
- 6) Snap freeze actin in liquid nitrogen at 10 mg/ml to maintain high biological activity.

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:

* Rhodamine actin protein (human platelet non-muscle)	Cat. # APHL
* 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
* 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
* Actin protein (bovine cardiac muscle, >99% pure)	Cat. # AD99
* Actin protein (chicken gizzard, >99% pure)	Cat. # AS99
* Pre-formed Actin Filaments (rabbit muscle, >99% pure)	Cat. # AKF99
* Rhodamine phalloidin	Cat. # PHDR1
* Actin Binding Protein Assay Kit	Cat. # BK001
* Actin Polymerization Biochem Kit	Cat. # BK003
* <i>In vivo</i> G-Actin / F-Actin Assay Kit	Cat. # BK037

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Rhodamine Muscle Actin (Rabbit muscle, > 99% pure)

Cat. # AR05

Lot # 034 Amount: 5 x 20 µg

Store at 4°C (desiccated) or at -70°C

Material

Purified rabbit muscle actin (Cat. # AKL99) has been modified to contain covalently linked rhodamine at random surface lysine residues. An activated ester of rhodamine is used to label the protein. The labeling stoichiometry has been determined to be 0.5 dyes per actin monomer. Rhodamine labeled rabbit muscle actin has an approximate molecular weight of 43 kDa, and is supplied as a pink lyophilized powder.

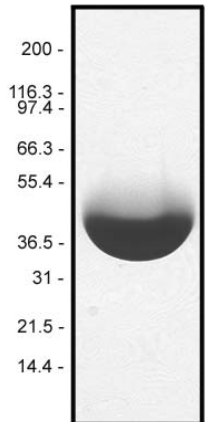
Storage and Reconstitution

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 2 µl of distilled water; it 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 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. Rhodamine 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. Rhodamine muscle actin was found to be >99% pure (see Figure 1).

Figure 1. Rhodamine Muscle Actin Protein Purity Determination. A 100 µg sample of rhodamine 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.



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Product Uses

- * *In vivo* actin polymerization studies (micro-injection into muscle cells)
- * *In vitro* motility studies using fluorescent F-actin and muscle myosins

Biological Activity Assays

1. Microinjection of rhodamine muscle actin into tissue culture cells

Reagents

- 1) Rhodamine muscle actin (Cat. # AR05)
- 2) General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM CaCl₂; Cat. # BSA01)
- 3) Tissue culture cells seeded on glass coverslips

Equipment

- 1) Microinjector apparatus

Method

- 1) Resuspend rhodamine muscle actin to 2 mg/ml with General Actin Buffer supplemented with 0.2 mM ATP and 1 mM DTT. Leave on ice for 1 h to depolymerize actin oligomers. Note: rhodamine muscle actin can be resuspended in 5 mM Tris-HCl pH 8.0, 10 μM MgCl₂ if microinjecting CaCl₂ is detrimental to your cells.
- 2) Centrifuge the muscle actin solution at 100,000 x g at 4°C for 10 min. Remove the top 90% of the supernatant to a new tube and load the microinjection needle by capillary action.
- 3) Microinject rhodamine muscle actin into cells maintaining positive pressure in the needle to prevent blockage. Note: salt in the tissue culture media and in the cell cytoplasm can result in actin polymerization within the needle.

2. Fluorescent imaging of *In vitro* polymerized rhodamine labeled actin filaments

Reagents

- 1) Rhodamine muscle actin (Cat. # AR05)
- 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) 10 μM phalloidin

Equipment

- 1) Fluorescent microscope with an excitation filter at 535 nm and emission filter at 585 nm
- 2) Digital CCD camera

Method

- 1) Resuspend rhodamine muscle actin to 0.5 mg/ml with General Actin Buffer supplemented with 0.2 mM ATP and 1 mM DTT. Add 1/10th the volume of Polymerization Buffer supplemented with 1 mM DTT and incubate at room temperature for 1 h.
- 2) Dilute the polymerized actin filaments 100 fold in 1x Polymerization Buffer containing 70 nM phalloidin and spot 1 μl into a drop of anti-fade solution (Cat. # BSM02) on a microscope slide.
- 3) Place a coverslips over the drop and remove excess liquid with a tissue.
- 4) Observe rhodamine labeled actin filaments with a fluorescent microscope.
- 5) A typical fluorescent image is shown in Figure 2.

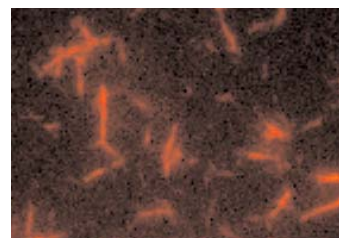


Figure 2. Fluorescent image of rhodamine muscle actin filaments. Rhodamine muscle actin was polymerized for 1 h, spotted onto a microscope slide and observed under a fluorescent microscope equipped with a Coolsnap digital CCD camera and 100x objective.

Quality Control

1. Polymerization spin down assay

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

Reagents

- 1) Rhodamine muscle actin (Cat. # AR05)
- 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) 100 mM ATP solution (Cat. # BSA04)
- 5) 1 M DTT solution
- 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 the rhodamine 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 microfuge 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.

2. Stoichiometry of Rhodamine Labeling

Reagents

- 1) Rhodamine muscle actin (Cat. # AR05)

Equipment

- 1) SPECTRAmax 250 microplate spectrophotometer (Molecular Devices Corp.)
- 2) UV-compatible microtiter plate

Method

- 1) Rhodamine muscle actin was resuspended to 0.13 mg/ml with Milli-Q water and added into well of a UV-compatible 96 well microtiter plate.
- 2) The absorbance was scanned every 10 nm between 250 and 650 nm.
- 3) The labeling stoichiometry was then calculated using the Beer-Lambert law:
 $A = \epsilon \times \text{path length} \times \text{concentration}$, where ϵ is the extinction coefficient for tetramethyl rhodamine in cm⁻¹M⁻¹(85,800).
- 4) The absorption scan is shown in Figure 3.