V. 1.1

Pyrene Muscle Actin
(Rabbit muscle, >99% pure)
Cat. # AP05

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

Material

Purified rabbit muscle actin (Cat. # AKL99) has been modified to contain covalently linked pyrene at the cysteine 374 residue. An N-(1-pyrene) iodoacetamide is used to label the actin protein. Pyrene labeling stoichiometry has been determined to be between 0.4-0.6 dyes per actin monomer. Pyrene labeled rabbit muscle actin has an approximate molecular weight of 43 kDa 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 is stable for 6 months when stored desiccated to <10% humidity at 4°C. The protein should be reconstituted to 20 mg/ml with 50 µl of cold distilled water; it 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 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. Pyrene muscle actin is a labile protein and should be handled with care. Diluted pyrene actin is stable for a maximum of 4 h at 4°C and should not be frozen. Avoid repeated freeze-thaw cycles and do not freeze below 20 mg/ml.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% gradient polyacrylamide gel. Pyrene muscle actin is >99% pure (see Figure 1).

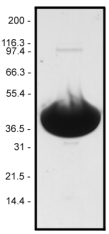

Figure 1. Pyrene Muscle Actin Protein Purity Determination. A 100 µg sample of pyrene muscle actin (molecular weight approx. 43 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined with the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The fluorescent signal of monomer pyrene actin is enhanced during its polymerization into filaments, making it an ideal tool for monitoring actin filament formation. Stringent quality control ensures that pyrene F-actin has a 7-12 fold fluorescent enhancement over non-polymerized pyrene G-actin.

Fluorescence enhancement

Reagents

1. Pyrene muscle actin (Cat. # AP05)
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)

Equipment

1. Fluorescence spectrophotometer with an excitation wavelength of 360 +/- 20 nm and an emission wavelength of 405 +/- 10 nm or 420 +/- 20 nm. Cytoskeleton recommends the SPECTRAFluor Plus from TECAN Austria GmbH or Gemini from Molecular Devices Inc.
2. Black polystrene 96 well assay plate (Costar, Cat. # 3915).

Method

1. Dilute pyrene muscle actin to 0.45 mg/ml with General Actin Buffer (Cat. # BSA01) supplemented with 0.2 mM ATP and 1 mM DTT. Leave on ice for 1 h to depolymerize actin oligomers.
2. Centrifuge at 14,000 rpm at 4°C for 30 min to remove residual nucleating centers.
3. Pipet 200 µl of the actin dilution into two wells of a black assay 96 well plate.
4. Pipet 200 µl of General Actin Buffer into two wells (control samples)
5. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 3 min to establish a baseline fluorescent measurement.
6. After 3 min add 20 µl of 10x Actin Polymerization Buffer (Cat. # BSA02) to each well and mix.
7. Return the plate to the spectrophotometer and read the fluorescence every 30 s for 1 h.
8. A typical polymerization fluorescent enhancement curve is shown in Figure 2.

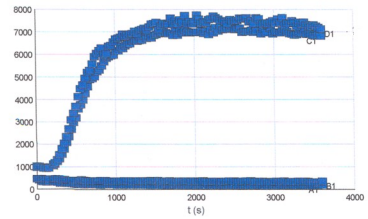

Figure 2. Fluorescence enhancement during pyrene actin polymerization. Pyrene muscle actin was polymerized in duplicate wells of a 96 well plate according to the method. The fluorescent signal was scanned every 30 s for 1 h. Polymerized pyrene F-actin shows a 10 fold fluorescent enhancement over non-polymerized pyrene G-actin and buffer control.

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Quality Control

The biological activity of pyrene 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 polymerized in this assay.

F-actin Polymerization Spin-down Assay

Reagents

1. Pyrene muscle actin (Cat. # AP05)
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. 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. Dilute the pyrene 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.

Stoichiometry of Pyrene Labeling

Reagents

1. Pyrene muscle actin (Cat. # AP05)

Equipment

1. SPECTRAmax 250 microplate spectrophotometer (Molecular Devices Corp.)
2. UV-compatible 96 well plate

Method

1. Pyrene muscle actin was diluted to 1.0 mg/ml in General Actin Buffer and added into single well of a UV-compatible 96 well plate.
2. The absorbancy was scanned every 10 nm between 250 and 500 nm.
3. The labeling stoichiometry was calculated using the Beer-Lambert law: A = ε x path length x concentration, where ε is the extinction coefficient for pyrene iodoacetamide in $cm^{-1}M^{-1}$(22,000).
4. The absorption scan for pyrene actin shown in Figure 3.

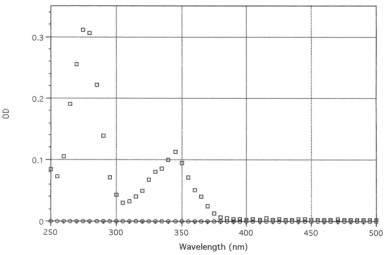

Figure 3. Absorption scan of pyrene muscle actin in solution. Pyrene muscle actin was diluted General Actin buffer in a microtiter plate well according to the method and its absorbance spectrum scanned between 250 and 500 nm. Labeling stoichiometry was calculated to be between 0.4-0.6 dyes per actin 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 Mg^{2+}.
3. Monomer actin will not polymerize at <2 mM K+, Na+, or in <0.05 mM Mg^{2+}.
4. Monomer actin is unstable below pH 6.5, or above pH 8.5.
5. Polymerized pyrene actin is more resilient to adverse conditions than monomeric actin. Therefore, pyrene actin is preferably stored in the polymerized form at 4°C for one to two days.
6. Snap freeze monomer pyrene actin in liquid nitrogen at 20 mg/ml to maintain high biological activity.

Product Uses

- Reagent for studying muscle actin polymerization *in vitro* (see fluorescence assay).
- Reagent for studying the affects of muscle actin binding proteins and drugs on actin polymerization *in vitro*.

Product Citations/Related Products

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