Pyrene Muscle Actin
(bovine cardiac muscle, >99% pure)
Cat. # CS-AP07
Lot: 023  Amount:  1 x 250 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information
Actin is a 43 kDa protein that is very highly conserved between species. There are three main actin isoforms (α, β and γ), which show >90% amino-acid (aa) homology between isoforms and >98% homology within members of a particular isotypic group. The majority of the isoform heterogeneity is located in the amino-terminal 30 residues. The amino-terminus of actin is located on the periphery of the double-helix in F-actin (1) and this site is also thought to interact with myosin (2).

Importantly, different actin isoforms have been shown to behave very differently in vitro and in vivo. Recent studies describe differential importances to form Filamentous-actin (F-actin) with the concomitant hydrolysis of ATP. F-actin is a double-helical filament as shown in Figure 1.

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 1) 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 2 mM Mg2+ and 50 mM KCl, but when these ions are absent, the CC is greater than 3.0 mg/ml. Thus, by altering the ionic type and strength one can alter the amount of polymer formed.

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 in Figure 1.

Material
Purified bovine cardiac muscle actin (Cat. # AD99) 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 0.25 dyes per actin monomer. (See Figure 4). Pyrene labeled rabbit muscle actin has an approximate molecular weight of 43 kDa and is supplied as a white lyophilized powder.

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. Pyrene muscle actin was found to be >90% pure (see Figure 3).

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 be polymerized in this assay.

Storage and Reconstitution
Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 6 months. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend each vial to 20 mg/ml with 12.5 µl cold Milli-Q water. The protein will then be in the following buffer: 5mM Tris-HCl pH 8.0, 0.2 mM CaCl2, 0.2 mM ATP, 5% (w/v) sucrose, and 1% (w/v) dextran.

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.
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 4-6 fold fluorescent enhancement over non-polymerized pyrene G-actin.

1. Fluorescence Enhancement
Reagents
1. Pyrene Cardiac Muscle Actin (Cat. # CS-AP07)
2. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM CaCl2) (Cat. # BSA01)
3. 10 x Polymerization Buffer (500 mM KCl, 20 mM MgCl2, 10 mM ATP) (Cat. # BSA02)

Method
1. Dilute pyrene muscle actin to 0.30 mg/ml with 0.833 ml General Actin Buffer supplemented with 0.2 mM ATP and 1 mM DTT.
2. Incubate at room temperature for 60 min to depolymerize actin oligomers that form during storage.
3. Centrifuge the protein in a 4°C microfuge at 14k rpm for 15 min to remove residual nucleating centers.
4. Pipette 200 µl of the actin solution into two wells of a black assay plate.
5. Pipette 200 µl of General Actin Buffer into two wells (control samples).
6. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 3 min to establish a baseline fluorescence measurement.
7. After 3 min add 20 µl of 10x Actin Polymerization Buffer (Cat. # BSA02) to each well and mix.
8. Return the plate to the spectrophotometer and read the fluorescence every 30 s for 1 h.
9. A typical fluorescent enhancement curve is shown in Figure 5.

![Fluorescence enhancement during pyrene actin polymerization](image)

**Figure 5: 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 4 to 6 fold fluorescent enhancement over non-polymerized pyrene G-actin and buffer control.

2. F-actin Polymerization Spin-down Assay Reagents
1. Pyrene Cardiac Muscle Actin (Cat. # CS-AP07)
2. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM CaCl2) (Cat. # BSA01)
3. 10 x Polymerization Buffer (500 mM KCl, 20 mM MgCl2, 10 mM ATP) (Cat. # BSA02)
4. 100 mM ATP solution (Cat. # BSA04)
5. 1M DTT solution
6. Precision Red Protein Assay Reagent (Cat. # ADV02)

Equipment
1. Microfuge at 4°C

1. Precision Red Protein Assay Reagent (Cat. # ADV02)
2. 1M DTT solution
3. 100 mM ATP solution (Cat. # BSA04)
4. Pyrene Cardiac Muscle Actin (Cat. # CS-AP07)
5. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM CaCl2) (Cat. # BSA01)
6. 10 x Polymerization Buffer (500 mM KCl, 20 mM MgCl2, 10 mM ATP) (Cat. # BSA02)
7. Centrifuge the protein in a 4°C microfuge at 14k rpm for 15 min to remove residual nucleating centers.
8. Return the plate to the spectrophotometer and read the samples for 3 min to establish a baseline fluorescence measurement.
9. After 3 min add 20 µl of 10x Actin Polymerization Buffer to each airfuge tube and mix well.
10. Incubate at room temperature for 1h.
11. Centrifuge the tubes at 100,000 x g for 1h to pellet the polymerized actin.
12. Remove the top 90% of the supernatant of each tube to a clean ultracentrifuge tube.
13. Add 20 µl (1/10th the volume) of 10 x Polymerization Buffer to each airfuge tube and mix well.
14. Incubate at room temperature for 1h.
15. Centrifuge the tubes at 100,000 x g for 1h to pellet the polymerized actin.
16. Remove the top 90% of the supernatant of each tube to a clean microcentrifuge tube.

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 >2 mM K+, Na+, and in >0.05 mM Mg2+.
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 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 µg/ml ampicillin and 10 µg/ml chloramphenicol is recommended. Cover in foil to stop photobleaching.

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

References

Product Citations / Related Products
For the latest citations and related products please visit www.cytoskeleton.com