PurSolutions
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PurSolutions LLC
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Charlottesville, VA 22901

DATASHEET: PURE ACTIN (>90%)

Catalog Number: 190101
Source: Rabbit Skeletal Muscle
Store Desiccated

Background:

Actin, a highly conserved cytoskeletal protein, is required for several essential eukaryotic processes including cell migration, muscle contraction, and cytokinesis. Actin (43 kDa) exists either as a free monomer termed G-actin (globular) or a linear polymer termed F-actin (filamentous). Given the asymmetry of G-actin, F-actin has inherent polarity with distinct "pointed" and "barbed" ends. Another critical feature of F-actin is its ability to treadmill, wherein G-actin is exchanged from both ends of the filament. This property emerges from the ATPase activity of G-actin and confers force-generating capabilities to F-actin. Mutations within actin and actin-associated proteins correlate with a variety of human diseases, including deafness and skeletal and cardiac myopathies.

Material:

Pure Actin is extracted from rabbit skeletal muscle using an optimized version of the method of Spudich and Watt (1971) and lyophilized by an adaptation of the method of Dráberová *et al*. (2010). The resulting product is >90% pure (Figure 1) and >80% polymerization competent (Figure 2). Possible contaminants include α-actinin (100 kDa, Figure 1). For actin >99% pure, see our Ultra-Pure Actin product (Cat. No. 160101). Pure Actin is supplied as a white powder. When reconstituted with ultrapure water to 9 mg/ml, the buffer conditions are 2 mM Tris-HCl, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1 mM DTT, and 0.25 M Trehalose, pH 8.0. Note that 1 mg actin is supplied as 3 mg powder, and reconstitution/dilution should be based on actin concentration.

Storage and Handling:

Store Pure Actin in a cool, dry environment. The product is stable under these conditions for 1 year. Reconstitute Pure Actin by resuspending in ice-cold ultrapure water to 9 mg/ml. Reconstituted Pure Actin can be maintained at 4°C for several weeks with the addition of 0.05% sodium azide. Alternatively, reconstituted Pure Actin can be aliquoted into experimental-sized batches, flash frozen in liquid Nitrogen, and stored at -80°C. Avoid repeated freeze-thaw cycles. When necessary, dilute reconstituted Pure Actin with Actin Working Buffer (Cat. No. 000102, 5 mM Tris-HCl and 0.2 mM $CaCl_2$, pH 8.0) supplemented with 0.2 mM ATP and 0.5 mM DTT. Note that 1 mg actin is supplied as 3 mg powder, and reconstitution/dilution should be based on actin concentration.

Activity:

When supplemented with KCl and $MgCl_2$, Pure Actin will polymerize into filaments when above its critical concentration. The recommended actin concentration for ensuring polymerization is 0.4 mg/ml.

Uses:
Pure Actin is supplied for use in cell-free experimental systems including structural, biochemical, and biophysical studies.

Polymerization Protocol:
Dilute Pure Actin to 0.4 mg/ml with Actin Working Buffer (Cat. No. 000102, 5 mM Tris-HCl and 0.2 mM $CaCl_2$, pH 8.0) supplemented with 0.2 mM ATP and 0.5 mM DTT. Incubate on ice for 1 hour followed by centrifugation at 14k rpm for 15 minutes at 4°C. Collect the supernatant and add 1/10 volume of Actin Polymer Buffer (10X; Cat. No. 000103; 500 mM KCl and 20 mM $MgCl_2$). Incubate at room temperature for 1 hour to polymerize. The resulting actin filaments can be stored at 4°C for several weeks in the presence of 0.05% sodium azide.

Technical Notes:
- store in a cool, dry environment
- maintain in the presence of ATP and DTT
- store at 4°C with 0.05% sodium azide upon reconstitution
- regard actin concentration and KCl and $MgCl_2$ addition when polymerizing

Figure 1: Pure Actin is >90% pure. Coomassie G250-stained protein gel of Pure Actin separated by SDS-PAGE. The actin appears as the majority species migrating at ~43 kDa. Possible contaminants include a-actinin (100 kDa) and are not in excess of 10%. Molecular weight markers (kDa) and loaded protein quantities are indicated.

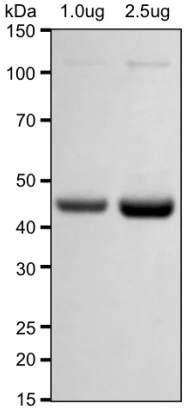
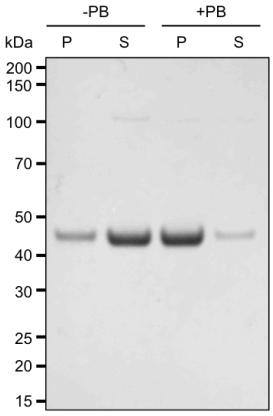


Figure 2: Pure Actin is >80% polymerization-competent. Pure Actin was polymerized in the absence (-PB) or presence (+PB) of Actin Polymer Buffer (10X, Cat. No. 000103; 50 mM KCl and 2 mM $MgCl_2$) followed by centrifugation at 48k rpm for 1 hour. Pellet (P) and supernatant (S) fractions were collected and subjected to SDS-PAGE and Coomassie G250-staining. >80% of Pure Actin was incorporated into filaments as determined by measuring the residual protein concentration in the supernatant fraction.



References:

1. Dráberová, E., Sulimenko, V., Sulimenko, T., Böhm, K., & Dráber, P. Recovery of tubulin functions after freeze-drying in the presence of trehalose. *Analytical Biochemistry.* **397,** 67–72 (2010).
2. Spudich, J.A. & Watt S. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem*. **246,** 4866-4871 (1971).