

DATASHEET: ULTRA-PURE ACTIN (>99%)

Catalog Number: 160101
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:

Ultra-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 >99% pure (Figure 1) and >90% polymerization competent (Figure 2). Ultra-Pure Actin is supplied as a white powder. When reconstituted with ultrapure water to 3 mg/ml, the buffer conditions are 2 mM Tris-HCl, 0.2 mM CaCl₂, 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 Ultra-Pure Actin in a cool, dry environment. The product is stable under these conditions for 1 year. Reconstitute Ultra-Pure Actin by resuspending in ice-cold ultrapure water to 3 mg/ml. Reconstituted Ultra-Pure Actin can be maintained at 4°C for several weeks with the addition of 0.05% sodium azide. Alternatively, reconstituted Ultra-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 Ultra-Pure Actin with Actin Working Buffer (Cat. No. 000102; 5 mM Tris-HCl and 0.2 mM CaCl₂, 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 MgCl₂, Ultra-Pure Actin will polymerize into filaments when above its critical concentration. The recommended actin concentration for ensuring polymerization is 0.4 mg/ml.

Uses:

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

Polymerization Protocol:

Dilute Ultra-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: Ultra-Pure Actin is >99% pure. Coomassie G250-stained protein gel of Ultra-Pure Actin separated by SDS-PAGE. The actin appears as a single species migrating at ~43 kDa. Molecular weight markers (kDa) and loaded protein quantities are indicated.

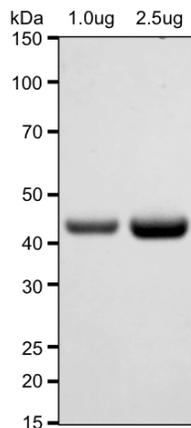
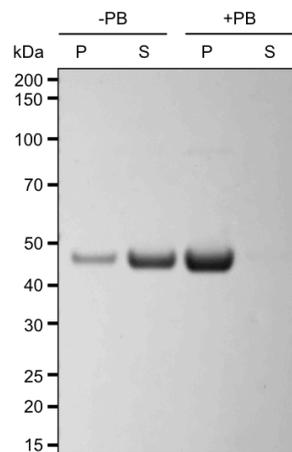


Figure 2: Ultra-Pure Actin is >90% polymerization-competent. Ultra-Pure Actin was polymerized in the absence (-PB) or presence (+PB) of Actin Polymer Buffer (10X, Cat. No. 000103; 50 mM KCl and 2 mM MgCl_2) followed by centrifugation at 48k rpm for 1 hour. Pellet (P) and supernatant (S) fractions were collected and subjected to SDS-PAGE and Coomassie G250-staining. >90% of Ultra-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).