DATASHEET

PurSolutions™

PURE ACTIN

Catalog Number	Mass	Quantity
190101 - 5 mg	15 mg total powder	5 mg actin protein
190101 - 10 mg	30 mg total powder	10 mg actin protein

STORE IN A COOL, DRY ENVIRONMENT

For research use only.

Shipping: shipped at ambient temperatures

Storage Conditions: store in cool, dry environment

Form: desiccated powder (1 mg actin protein is supplied as 3 mg powder with extra mass attributed to trehalose, a lyoprotectant)

Source: rabbit skeletal muscle

Molecular Weight: ~43 kDa

Purity: >90% (SDS-PAGE)

Buffer Conditions Upon Reconstitution: 2 mM Tris-HCl, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1 mM DTT, and 0.25 M Trehalose (pH 8.0)

Shelf Life: check product label for expiration date

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 actin protein is >90% pure (Figure 1) and >80% polymerization competent (Figure 2). Possible contaminants include α-actinin (100 kDa). For actin protein of >99% purity, 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 protein is supplied as 3 mg powder (extra mass attributed to trehalose, a lyoprotectant), and reconstitution/dilution should be based on the actin protein concentration.

Storage and Handling

Store Pure Actin in a cool, dry environment. The product is stable under these conditions for 1 year. Reconstitute the lyophilized actin protein by resuspending in ice-cold ultrapure water to 9 mg/ml. Dilute to 0.4 mg/ml with Actin Working Buffer (Cat. No. 000102, 5 mM Tris-HCl, 0.2 mM $CaCl_2$, pH = 8.0) and add ATP to 0.2 mM and DTT to 0.5 mM. Incubate on ice for 1 hour and mix occasionally with gentle vortexing. Clarify the reconstituted actin to remove any protein aggregates by centrifuging at 14k rpm (21k x g) for 15 minutes at 4°C. Add sodium azide to 0.05% and store at 4°C.

Reconstituted Pure Actin can be maintained at 4°C for several weeks with the addition of 0.05% sodium

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azide. If desired, reconstituted Pure Actin can be aliquoted into smaller experimental batches, frozen in liquid Nitrogen, and stored at -80°C. Avoid repeated freeze-thaw cycles. Note that 1 mg actin is supplied as 3 mg powder (extra mass attributed to trehalose, a lyoprotectant), and reconstitution/dilution should be based on the actin protein concentration. View detailed storage and handling instructions at https://puresoluble.com/storage-and-handling-pure-actin/.

Activity and Applications

Pure Actin will polymerize into filamentous F-actin when supplemented with KCl and $MgCl_2$, and kept above its critical concentration. Pure Actin is suitable for use in a variety of cell-free experimental applications, and polymerization activity is detectable in fluorescence microscopy assays, turbidity assays, and ATPase assays. Visit www.PureSoluble.com/protocols for common actin polymerization protocols.

- antibody generation
- drug discovery by high-throughput screening
- *in vitro* biochemical and biophysical approaches
- structural analysis by X-ray crystallography and electron microscopy

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 α-actinin (100 kDa) and are not in excess of 10%. Molecular weight markers and loaded protein quantities are indicated.

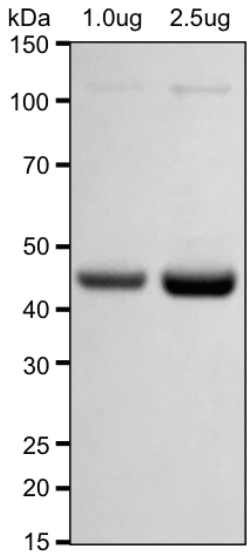
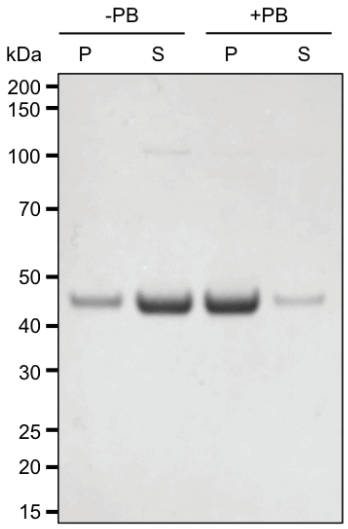


Figure 2: Pure Actin is >80% polymerization-competent.
Pure Actin polymerized in the absence (-PB) or presence (+PB) of Actin Polymer Buffer (10X, Cat. No. 000103; 50 mM KCl and 2 mM $MgCl_2$) followed by centrifugation at 48k rpm (100k x g) for 1 hour. Pellet (P) and supernatant (S) fractions were collected 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).