The Protein Experts

Cytoskeleton

Version 2.0

Profilin Protein
(Human recombinant)
Cat. # PR01
Lot # 030 Amount: 1 x 50 µg
Store at 4˚C (desiccated) or at -70˚C

Material

Human profilin 1 protein has been produced and purified from a bacterial expression system. The recombinant protein contains six histidine residues at its carboxy- terminus (His-tag) and has an approximate molecular weight of 21 kDa. Profilin is a small globular actin binding protein capable of binding actin monomers with micromolar affinity at a stoichiometry of 1:1 (1, 2). The net result of profilin binding to actin is an inhibition of actin polymerization. Profilin is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 1 mg/ml by the addition of 50 µl of Milli-Q water. The protein will be in the following buffer: 10 mM Tris pH 7.5, 10 mM NaCl, 0.2 mM ATP, 5% (w/v) sucrose and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is recommended that the protein solution be aliquoted into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70˚C. The protein is stable for 6 months if stored at -70˚C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4˚C desiccated (<10% humidity) for 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% gradient polyacrylamide gel. Profilin protein was determined to be 80% pure (see Figure 1).

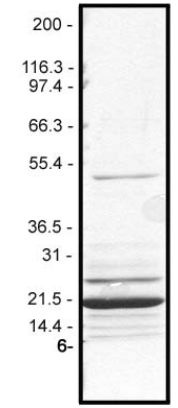

Figure 1. ***Profilin Protein Purity Determination***. A 10 µg sample of profilin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of profilin can be determined by its ability to inhibit actin polymerization. G-actin is incubated with and without profilin before the addition of actin polymerization buffer. F-actin is separated from G-actin by centrifugation and the proportion of actin in the supernatant (G-actin) versus the pellet (F-actin) is compared to a control reaction without profilin. Stringent quality control ensures that profilin (5 µg) can inhibit actin (10 µg) polymerization by 50%.

Reagents

1) Profilin protein (50 µg, Cat. # PR01)
2) Rabbit muscle actin (250 µg Cat. # AKL99-A)
3) General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$; Cat. # BSA01)
4) 10x Actin Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP; Cat. # BSA02)

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) Protein electrophoresis apparatus

Method

1) Resuspend the profilin protein to 1.0 mg/ml in cold General Actin Buffer. Keep on ice.
2) Centrifuge the profilin protein at 12k rpm at 4˚C for 10 min to pellet any denatured protein.
3) Resuspend the rabbit muscle actin to 1.0 mg/ml with cold General Actin Buffer. Incubate on ice for 30 min to depolymerize actin oligomers that form during storage.
4) Centrifuge the protein in a 4˚C microfuge at 14k rpm for 15 min. Transfer the clarified supernatant to a new microfuge tube. Keep on ice
5) Label three centrifuge tubes (1, 2 and 3) and place on ice.
6) Add 10 µg of G-actin to tubes 2 and 3. Keep on ice.
7) Add 5 µg of profilin protein to tubes 1 and 3. Keep on ice.
8) Bring the volume of each tube to 50 µl with General actin buffer.
9) Incubate all tubes at 30°C for 30 min.
10) Add 1/10th the volume of Actin Polymerization Buffer to each tube and mix well. Incubate at room temperature for 30 min to polymerize actin.
11) Centrifuge the tubes at 100,000 x g for 1 h to pellet the F-actin.
11) Remove the supernatant of each tube to a clean labeled (1S, 2S and 3S) microfuge tubes. Avoid touching the bottom of the tube or disturbing the pellet material.
12) Add 10 µl of 5x Laemmli-reducing sample buffer to each supernatant sample.
13) Resuspend the pellet fraction (F-actin) in each ultracentrifuge tube with 60 µl of Laemmli-reducing sample buffer. Transfer to labeled microfuge tubes (1P, 2P and 3P).
14) Load the supernatant and pellet samples on and SDS-gel and electrophoresis. Stain with Coomassie Blue.
15) The results of a typical actin polymerization inhibition assay is shown in Figure 2.

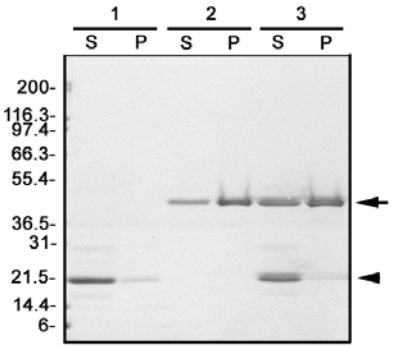

Figure 2. ***Actin Polymerization Inhibition Assay.*** The ability of profilin to inhibit actin polymerization was assessed by SDS-PAGE of proportionally loaded supernatant (S) and pellet (P) fractions from G-actin (10 µg, arrow) incubated with and without profilin (5 µg, arrowhead) according to the assay method. In the absence of profilin approx. 80% of the actin protein (43 kDa) is found in pellet fraction as F-actin (lane 2). When G-actin is incubated with profilin prior to polymeization, only 50% of actin is found as F-actin in the pellet (P), the other 50% remains as G-actin in the supernatant (S, lane 3). Lane 1, profilin protein alone. Mark12 molecular weight markers are from Invitrogen.

Product Uses

* Positive control for the studying the G-actin binding proteins
* Investigation of the the effect of actin binding proteins (ABP's) on actin dynamics

References

1) Carlsson et.al. 1977. *J. Mol. Biol.* 115:465-483.
2) Larsson et. al. 1988. *Biochim. Biophys. Acta.* 953:95-105.

Related Products

Cytoskeleton Inc. offers the widest range of actin and actin associated products currently available. We specialize in producing highly purified proteins with high biological activity. These include the actin Biochem Kits, actin associated proteins, and other reagents for actin based assays. Included in the actin product line are:

* Actin Binding Protein Assay Kit (muscle actin)	Cat. # BK001
* Actin Binding Protein Assay Kit (non-muscle actin)	Cat. # BK013
* Actin Polymerization Biochem Kit	Cat. # BK003
* *In vivo* G-Actin / F-Actin Assay Kit	Cat. # BK037
* Heavy meromyosin protein (rabbit skeletal muscle)	Cat. # MH01
* Myosin II protein (rabbit skeletal muscle)	Cat. # MY02
* Alpha actinin protein (rabbit skeletal muscle)	Cat. # AT01
* Arp2/3 protein complex (bovine brain)	Cat. # RP01
* Cofilin protein (rabbit skeletal muscle)	Cat. # CF01
* Gelsolin protein (human recombinant)	Cat. # HPG6
* Actin protein (rabbit skeletal muscle, >99% pure)	Cat. # AKL99
* Actin protein (rabbit skeletal muscle, >95% pure)	Cat. # AKL95
* Actin protein (human platelet, non-muscle, >99% pure)	Cat. # APHL99
* Actin protein (human platelet, non-muscle, >95% pure)	Cat. # APHL95
* Actin protein (bovine cardiac muscle, >99% pure)	Cat. # AD99