

Profilin Protein
(Human recombinant)
Cat. # PR01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

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 μ l 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).

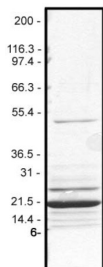


Figure 1. Profilin Protein Purity Determination. A 10 μ g sample of profilin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision RedTM Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers 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 μ g) can inhibit actin (10 μ g) polymerization by 50%.

Reagents

1. Profilin protein (50 μ g, Cat. # PR01)
2. Rabbit muscle actin (250 μ g 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 adaptors (Cat. # 356860), or other ultracentrifuge capable of centrifuging 200 μ l 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 μ g of G-actin to tubes 2 and 3. Keep on ice.
7. Add 5 μ g of profilin protein to tubes 1 and 3. Keep on ice.
8. Bring the volume of each tube to 50 μ l 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.

12. 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.
13. Add 10 μ l of 5x Laemmli-reducing sample buffer to each supernatant sample.
14. Resuspend the pellet fraction (F-actin) in each ultracentrifuge tube with 60 μ l of Laemmli-reducing sample buffer. Transfer to labeled microfuge tubes (1P, 2P and 3P).
15. Load the supernatant and pellet samples on and SDS-gel and electrophoresis. Stain with Coomassie Blue.
16. The results of a typical actin polymerization inhibition assay is shown in Figure 2.

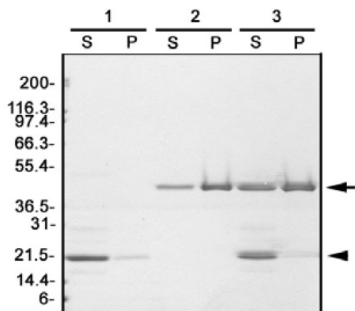


Figure 2. Actin Polymerization Inhibition Assay. The ability of profilin to inhibit actin polymerization was assessed by SDS-PAGE of proportionally loaded supernatant (S) and pellet (P) fractions from G-actin (10 μ g, arrow) incubated with and without profilin (5 μ g, arrowhead) according to the assay 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 polymerization, 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 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.

Product Citations/Related Products

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