V.2.0

Profilin-1 Protein

(Human recombinant)
Cat.# PR02

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 is untagged and has an approximate molecular weight of 15 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). Depending on conditions an molar ratios of actin to profilin, profilin can act to enhance or inhibit 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 either the addition of 100 µl of Milli-Q water for 100 µg of PR02-A, 500 µl of Milli-Q water for 500 µg of profilin PR02-B, or 1 ml of Milli-Q water for 1 mg of PR02-XL. The protein will be in the following buffer: 10 mM Tris pH 8.0, 1mM EDTA, 1mM DTT, 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 ≥ 95% pure (**Figure 1**).

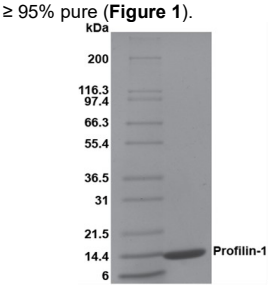

Figure 1. Profilin Protein Purity Determination. A 10 µg sample of profilin protein was separated by electrophoresis in a 4- 20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision RedTM Protein Assay Reagent (Cat.# ADV02). SeeBlue pre-stained standard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

Biological Activity Assays
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 (15 µg) can inhibit actin (10 µg) polymerization by ≥60% (Figure 2).

Reagents
1. Profilin Protein (100 µg, Cat. # PR02-A)
2. Rabbit muscle actin (250 µg Cat. # AKL99-A)
3. General Actin Buffer (5 mM Tris-HCl pH8.0, 0.2 mM $CaCl_2$; Cat. # BSA01)
4. 10x Actin Polymerization Buffer (500mM KCl, 20mM $MgCl_2$, 10mM 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: Actin Binding Assay
1. Resuspend the Profilin-1 protein to 1.0 mg/ml in cold General Actin Buffer. Keep on ice.
2. Centrifuge the Profilin-1 protein at 14k rpm at 4°C for 10min to pellet any denatured protein.
3. Resuspend the rabbit muscle actin to 1 mg/ml with cold General Actin Buffer. Incubate on ice for 1hr to depolymerize actin oligomers that form during storage.
4. Centrifuge the actin in a 4°C microfuge at 14k rpm for 15min. 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 15 µg of profilin-1 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 30min.
10. Add 1/10th the volume of Actin Polymerization Buffer to each tube and mix well. Incubate at room temperature for 1 h 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 clean labeled (1S, 2S, and 3S) microfuge tubes. Avoid touching the bottom of the tube or disturbing the pellet material.
13. Add 10 µl of 5x Laemmli-reducing sample buffer to each supernatant sample.
14. Resuspend the pellet fraction (F-actin) in each ultracentrifuge tube with 50 µl 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 are shown in Figure 2.

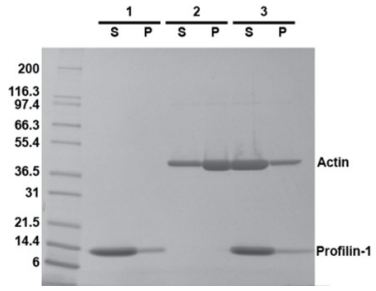

Figure 2. Actin Polymerization Inhibition Assay. The ability of profilin to inhibit actin polymerization was assessed by SDS-PAGE of proportionally loaded supernatant (S) and pellet (P) fractions from G-actin incubated with and without profilin-1 according to the assay method. In the absence of profilin-1, approx. 80% of the actin protein (43 kDa) is found in the pellet fraction as F-actin (P, lane 2). When G-actin is incubated with profilin prior to polymerization, only 20% approx. of actin is found as F-actin in the pellet (P, lane 3), while the other 80% remains as G-actin in the supernatant (S, lane 3). Lane 1, profilin-1 protein alone. Mark12 molecular weight markers are from Invitrogen.

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Method: Actin Polymerization Assay

Reagents

1. Profilin protein (2x1 mg, Cat. # PR02-XL)
2. Rabbit muscle actin (1 mg Cat. # AKL95-B)
3. Pyrene labeled actin (Cat # AP05)
4. Polymerization buffer 1.5x stock: 7.5 mM Tris pH 7.5, 75 mM KCl. 3 mM $MgCl_2$, 1.5 mM EGTA, 0.15 mM CaCl, 0.75 mM DTT, 0.3 mM ATP [add fresh from a 100 mM stock pH 7.0, immediately prior to use]
5. Supplemented H_2O stock: 0.2 mM ATP and 1 mM DTT
6. Arp2/3 protein complex (Cat. # RP01)
7. VCA domain-GST fusion (Cat # VCG03)

Equipment

1. Fluorimeter with an excitation wavelength of 350 or 360 ± 20 nm and an emission wavelength of 407 or 410 ± 10 nm or 420 ± 20 nm .
2. Black polystyrene 96 well assay plate (Costar, Cat. # 3915).
3. Ultracentrifuge capable of centrifuging at 50,000 x g.

Method: Actin Polymerization Assay

1. Resuspend and dilute both pyrene labeled (Cat. # APO5) and non-labeled (Cat. # AKL95-B) muscle actin to 0.2 mg/ml with supplemented H_2O. Leave on ice for 1 h to depolymerize actin oligomers.
2. Dilute pyrene labeled (Cat. # APO5) and non-labeled (Cat. # AKL95-B) muscle actin stocks to 1:1.
3. Centrifuge the actin at 50,000 x g at 4°C for 30 min to remove residual nucleating centers.
4. Pipette the top 80% of both actin supernatants into one new microfuge tube on ice.
5. Prepare fresh supplemented H_2O and mix it with the entire actin supernatant from step 4 to make a 1:1 ratio to yield a 0.1 mg/ml actin stock.
6. Dilute the Arp2/3 complex (Cat. # RP01) to 0.3 mg/ml in Milli-Q H_2O.
7. Resuspend one tube of VCA domain protein (Cat. # VCG03) to 1 mg/ml in H_2O.
8. Dilute the 1 mg/ml VCA domain protein stock to 0.125mg/ml.
9. Dilute Profilin-1 protein stock (Cat. # PR02) to 20 mg/ml in H_2O.
10. Add the following components to the 96 well assay plate:

Well	1.5x Poly Buffer (µl)	Arp2/3 (µl)	VCA domain (µl)	PR02 (µl)
A1	200	0	0	0
B1	200	0	0	0
C1	200	0	5	0
D1	200	0	5	0
E1	200	0	0	5.6
F1	200	0	0	5.6
G1	200	0	0	11.2
H1	200	0	0	11.2
A2	200	2	0	0
B2	200	2	0	0
C2	200	2	5	0
D2	200	2	5	0
E2	200	2	5	5.6
F2	200	2	5	5.6
G2	200	2	5	11.2
H2	200	2	5	11.2

11. Using a multi-channel pipet, add 100 µl of diluted actin to wells A1-H2 of the assay plate. Note: Do not introduce air bubbles into the wells.
12. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 1 h.
13. In the assay described above, actin is present at a final concentration of 0.8 µM, Arp2/3 complex at 10 nM, VCA domain at 400 nM, and Profilin-1 at 25 µM and 50 µM.
14. Results for a typical actin polymerization assay is shown in Figure 3.

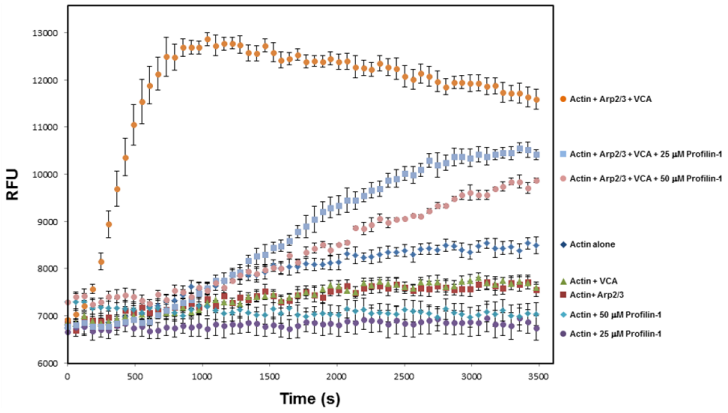

Figure 3. Profilin-1 Inhibits Branched Polymerization of Actin Filaments by the Arp2/3 Complex and the VCA Domain of WASP. Actin polymerization was carried out as described in the method; all reactions contain a 1:1 ratio of pyrene- and non-labeled- actin. Actin in the presence of both Arp2/3 and VCA shows enhancement of actin nucleation. Upon the addition of Profilin-1, the steep nucleation phase provided by both Arp2/3 and VCA is delayed and greatly reduced. The addition of Profilin-1 to actin also decreases the rate of actin polymerization when compared to actin alone. The mean values for each time point are from at least 3 independent experiments. Errors are standard error of the mean n ≥ 3.

Product Uses

- Inhibiting the formation of branched actin filaments.
- Positive control for the studying the G-actin binding proteins.

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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