V. 1.1

Cofilin Protein
(Human recombinant)
Cat. # CF01

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

Material

The human cofilin protein (isotype 1) has been produced in a bacterial expression system. The recombinant protein has a molecular weight of approximately 21 kDa, and does not contain a protein purification tag. Recombinant cofilin has been purified by ion exchange chromatography. Cofilin is one member of a large group of proteins characterized as "actin binding proteins"(ABPs). Cofilin is an essential cellular protein that can bind the barbed end of actin. In the cell, cofilin acts in concert with other regulatory proteins to mediate the response of the actin cytoskeleton to extracellular signals. In vertebrates, cofilin is regulated by pH (1), phosphorylation (2) and phosphoinisitides (3). Recombinant cofilin 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 5 mg/ml by the addition of 20 µl of distilled water. The protein will be in the following buffer; 10 mM Tris pH 8.0, 10 mM NaCl, 5% sucrose and 1% dextran. In order to maintain high biological activity of the protein, it is recommended that the protein solution be supplemented with DTT to 1 mM, 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. Cofilin protein was determined to be 95% pure (see Figure 1).

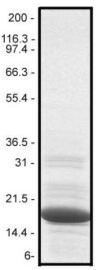

Figure 1. Human Cofilin Protein Purity Determination. A 20 µg sample of recombinant cofilin protein (molecular weight approx. 21 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of recombinant cofilin can be determined from its ability to bind and sever F-actin in a pH dependent manner. Below pH 7.0 cofilin binds to F-actin in a 1:1 molar ratio of cofilin to actin monomer in the filament. Above pH 7.0 cofilin will sever actin filaments and bind actin monomer in a 1:1 molar ratio. A standard biological assay for monitoring the actin binding and severing activity of cofilin consists of SDS-PAGE analysis of F-actin/cofilin spin down assays performed at pH 6.8 and 8.0.
Stringent quality control ensures that at pH 6.8 only 20% of cofilin and actin are found in the supernatant, and that a 1:1 molar ratio of cofilin to actin protein is present in the pellet. Furthermore, at pH 8.0 approximately 80% of cofilin and actin are found in the supernatant due to the F-actin severing activity of cofilin.

Reagents

1. Recombinant human cofilin protein (Cat. # CF01).
2. Rabbit muscle actin at 99% purity (Cat. AKL99)
3. 10 mM Tris-HCl pH 6.8.
4. 10 mM Tris-HCl pH 8.0.
5. Actin Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP, Cat. # BSA02)
6. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, Cat. # BSA01)

Equipment

1. 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.
2. SDS-PAGE apparatus

Method

1. Resuspend human cofilin (Cat. # CF01) to 5 mg/ml with distilled water. Keep on ice.
2. Dilute rabbit muscle actin (Cat. # AKL99) to 2.0 mg/ml with General Actin Buffer. Incubate on ice for 30 min to depolymerize any existing actin oligomers.
3. Polymerize the actin into filaments with the addition of 1/10th the volume of Actin Polymerization Buffer (Cat. # BSA02). Incubate at room temperature for 1 h.
4. Combine equimolar amounts of F-actin and cofilin into 200 µl of 10 mM Tris pH 6.8. For example, 1 nmol (40 µg) of F-actin was incubated with 1 nmol (20 µg) of cofilin.
5. Combine equimolar amounts of F-actin and cofilin into 200 µl of 10 mM Tris pH 8.0.
6. Add 40 µg of F-actin to 200 µl of 10 mM Tris pH 6.8 and to 10 mM Tris pH 8.0. These tubes will be the F-actin spin down controls.

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7. Add 20 µg of cofilin to 200 µl of 10 mM Tris pH 6.8 and to 10 mM Tris pH 8.0. These tubes will be the cofilin spin down controls.
8. Incubate the experimental and control tubes for 30 min at room temperature.
9. Centrifuge the tubes at 100,000 x g for 1 h to pellet F-actin and any associated cofilin protein.
10. Analyze the supernatant and pellet samples by SDS-PAGE and scanning densitometry.
11. Typical assay results are shown in Figure 2.

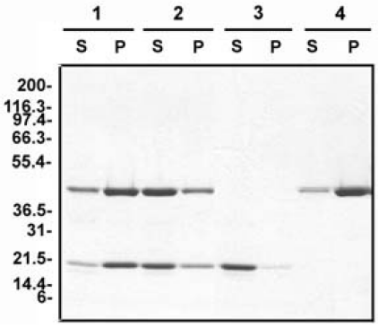

Figure 2. The pH Dependent Actin Binding and Severing activity of Human Cofilin. Human cofilin protein (20 µg) was incubated with 40 µg of F-actin in Tris pH 6.8 and Tris pH 8.0 as described in the method. Supernatant (S) and pellet (P) samples were then analyzed by SDS-PAGE and Coomassie Blue staining after a 100,000 x g spin. 1, Samples from pH 6.8 incubation. 2, Samples from pH 8.0 incubation. 3, Samples from cofilin alone incubation. 4, Samples from F-actin alone incubation. Note that at pH 6.8, 80% of actin is found in the pellet along with a 1:1 molar ratio of cofilin, whereas at pH 8.0, F-actin has been severed by cofilin resulting in 80% of the actin being found in the in the supernatant along with cofilin. Mark12 molecular weight markers are from Invitrogen.

Product Uses

- Studies of cofilin binding and severing activities
- Control protein for actin binding protein studies

References

1. Yonezawa, N., et al. 1985. *J. Biol. Chem*. 260:14410-14412
2. Moriyama, K., et al. 1996. *Genes Cells*. 1: 73-86.
3. Yonezawa, N., et al. 1990. *J. Biol. Chem*. 265:8382-8386

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