Fascin-1 Protein: wild-type
(Human recombinant)
Cat. # CS-FSC01
Lot # 011 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

V. 3.1

Material
The wild-type human fascin 1 protein has been produced in a bacterial expression system. The recombinant protein contains no tag. The molecular weight of fascin is approximately 54 kDa and it is supplied as a white lyophilized powder.

Storage and Reconstitution
Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with the addition of 20 µl of Milli-Q water (100 µg size). When reconstituted, the protein will be in the following buffer: 20 mM Tris pH 8.0, 150 mM NaCl, 2 mM $CaCl_2$, 5% (w/v) sucrose, and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment-sized" amounts, snap frozen in liquid nitrogen, and stored at -70°C. The protein is stable for six 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 one year.

Purity
Protein purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. Fascin 1 protein was determined to be >95% pure. (see Figure 1).

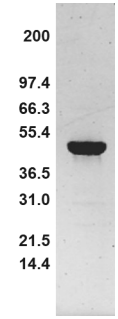

Figure 1. Fascin 1 Protein Purity Determination. A 10 µg sample of recombinant fascin 1 protein (molecular weight approx. 54 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Life Technologies Inc.

Biological Activity Assay
The biological activity of fascin 1 can be determined from its actin-bundling activity. The F-actin bundling assays with fascin 1 utilize polymerizing monomeric G-actin (Cat. # AKL-99) for either low-speed centrifugation assay or fluorescence microscopy. The F-actin is labeled with Acti-Stain™ 488 phalloidin (Cat. # PHDG1) for fluorescence microscopy.

Low-Speed Centrifugation Actin-Bundling Assay

Reagents
1. Recombinant fascin 1protein (Cat.# CS-FSC01)
2. Actin protein (Cat.# AKL99-A)
3. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$; Cat. # BSA01)
4. 10x Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP; Cat. # BSA02)
5. 5. 8 M urea in 10 mM Tris-HCl pH 7.5

Equipment
1. Table-top centrifuge capable of spinning 10,000 x g.

Method
1. Gently resuspend rabbit muscle actin (Cat. # AKL99-A) to 1 mg/ ml with 250 µl of ice cold General Actin Buffer supplemented with 0.2 mM ATP and 1.0 mM DTT and mix well but gently.
2. Incubate on ice for 60 min to depolymerize actin oligomers that form during storage.
3. Centrifuge the actin in a 4°C microfuge at 14k rpm for 15 min, and collect the supernatant into a fresh tube on ice.
4. Gently resuspend fascin 1 to 1 mg/ml by using 100 µl of ice cold General Actin Buffer supplemented with 0.2 mM ATP and 1.0 mM DTT, and place on ice.
5. Label three tubes A) F-actin alone, B) fascin 1 alone, and C) actin plus fascin 1. Add the following components to the labeled tubes.

Tube	General Actin Buffer (Cat. # BSA01)	Actin (Cat. # AKL99)	10x Polymerization Buffer (Cat. # BSA02)
A - Actin alone	35 µl	10 µl	5 µl
B - Fascin1 alone	42.5 µl	0 µl	5 µl
C - Actin + Fascin 1	32.5 µl	10 µl	5 µl

6. Incubate all reactions for 1 h at room temperature.
7. To tubes B and C, add 2.5 µl of fascin 1 (Cat. # CS-FSC01), mix gently, and incubate for 30 mins at room temperature.
8. Centrifuge all tubes at 10,000 x g for 20 mins at room

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temperature.

9. Pipette off 45 ul supernatants into fresh tubes on ice (Tip: The pellets are small to invisible so use a marker to circle the area that the pellet should be and avoid that area while pipetting off the supernatant).
10. Add 9 ul of 6x SDS-PAGE loading buffer to each supernatant tube and prepare for gel loading.
11. Resolubilize pellets in 49 ul of 1x SDS_PAGE loading buffer.
12. Supernatant and pellet fractions are analyzed and visualized by SDS-PAGE and Coomassie Blue staining (Figure 2).

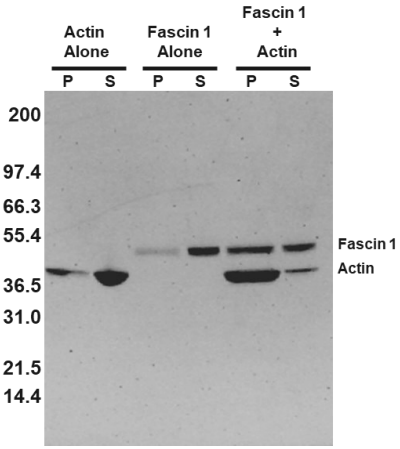

Figure 2. Low-Speed Centrifugation Actin-Bundling Assay with Fascin 1. Pellet and supernatant for each reaction were loaded equally on a 4-20% polyacrylamide gradient gel and the proteins were visualized by Coomassie Blue staining.

Fluorescence Microscopy Actin-Bundling Assay

Reagents

1. Recombinant fascin 1 protein (Cat.# CS-FSC01)
2. Actin protein (Cat.# AKL99-A)
3. General Actin Buffer (5 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$; Cat. # BSA01)
4. 10x Polymerization Buffer (500 mM KCl, 20 mM $MgCl_2$, 10 mM ATP; Cat. # BSA02)
5. Acti-stain™ 488 Phalloidin (Cat. # PHDG1)

Equipment

1. Fluorescence microscope with excitation filter at 450-480 +/- 20 nm and emission filter at 535 +/- 20nm and 63X—100X oil immersion lens.
2. Digital CCD camera.

Method

1. Resuspend rabbit muscle actin (Cat. # AKL99-A) to 1 mg/ ml with 250 µl of General Actin Buffer supplemented with 0.2 mM ATP and 1.0 mM DTT. Mix well and leave on ice for 1 h.
2. Incubate on ice for 60 min to depolymerize actin oligomers that form during storage.
3. Centrifuge the actin in a 4°C microfuge at 14k rpm for 15 min, and collect the supernatant into a fresh tube on ice.
4. Label two tubes A) actin alone and B) actin plus fascin 1. Add the following components to labeled tubes.

Tube	General Actin Buffer (Cat. # BSA01)	Actin (Cat. # AKL99)	10x Polymerization Buffer (Cat. # BSA02)
A - Actin alone	175 µl	50 µl	25 µl
B - Actin + Fascin 1	162.5 µl	50 µl	25 µl

5. Incubate all reactions for 1 h at room temperature.
6. To tube B add 12.5 µl of fascin 1, mix gently and incubate for 30 mins at room temperature.
7. Label actin by adding 5 µl of Acti-stain™ 488 Phalloidin to each tube.
8. Apply each sample to a coated 22 mm coverslip (collagen or poly-D-lysine) and incubate for 1h at room temp in the dark.
9. Mount coverslip to a glass slide and image with a fluorescence microscope.

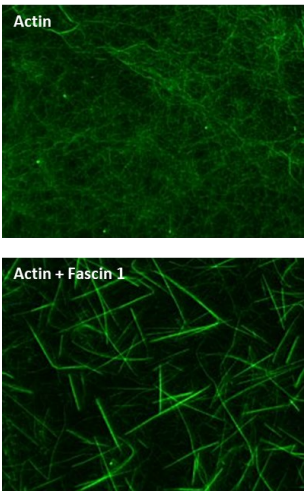

Figure 3. Fluorescence Microscopy Images of Actin-Bundling Assay with Fascin 1. Legend: Actin filaments alone and with fascin 1 stained with Acti-stain™ 488 Phalloidin as described in the method. Actin filaments were observed under a fluorescent microscope with a 480Ex/535Em filter set, a digital CCD camera, and 63x objective.

Product Uses

- Study actin-bundling activity.
- Identification of compounds that inhibit actin and fascin 1 actin-bundling activity.
- Biochemical characterization of fascin 1 protein interactions
- Western blot standard

Product Citations/Related Products

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