

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
* F-Actin Visualization Kit	Cat. # BK005
* <i>In vivo</i> G-Actin / F-Actin Assay Kit	Cat. # BK037
* Heavy meromyosin protein (rabbit skeletal muscle)	Cat. # MH01
* Myosin II protein (rabbit skeletal muscle)	Cat. # MY02
* Myosin protein (bovine cardiac muscle)	Cat. # MY03
* Alpha actinin protein (rabbit skeletal muscle)	Cat. # AT01
* Arp2/3 protein complex (bovine brain)	Cat. # RP01
* Cofilin protein (rabbit skeletal muscle)	Cat. # CF01
* Profilin protein (human platelet)	Cat. # PR01
* Actin protein (rabbit skeletal muscle, >99% pure)	Cat. # AKL99
* Actin protein (rabbit skeletal muscle, >95% pure)	Cat. # AKL95
* Pre-formed F-Actin (rabbit skeletal muscle)	Cat. # AKF99
* 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
* Actin protein (chicken gizzard smooth muscle, >99% pure)	Cat. # AS99
* Biotin actin protein (human platelet, non-muscle)	Cat. # APHB
* Biotin actin protein (rabbit skeletal muscle, >99% pure)	Cat. # AB07
* Pyrene actin protein (rabbit skeletal muscle, >99% pure)	Cat. # AP05
* Rhodamine actin protein (rabbit skeletal muscle)	Cat. # AR05
* Rhodamine actin protein (human platelet, non-muscle)	Cat. # APHR



Plasma Gelsolin (Human recombinant)

Cat. # HPG6

Lot # 001 Amount: 4 x 20 µg

Store at 4°C (desiccated) or at -70°C

Material

The human plasma gelsolin isoform has been purified from a bacterial expression system. The recombinant protein is tagged with six histidine residues (His-tag) at its amino terminus and has a total molecular weight of approx. 95 kDa. Plasma gelsolin differs from cytoplasmic gelsolin in that it is secreted from the cells and contains a 25 amino acid N-terminal extension (1).

Recombinant plasma gelsolin (20 µg of protein) is supplied as a white lyophilized powder.

Min. amount per tube	Actual amount per tube	Minimum required specific activity per tube	Actual HPG6 Lot 001 specific activity per tube
20 µg	23 µg	20 U/tube	24 U/tube

See Unit definition below on page 3.

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 20 µl of Milli-Q water. The protein will be in the following buffer: 10 mM Tris pH 7.5, 10 mM NaCl, 0.1 mM MgCl₂, 1% (w/v) sucrose and 0.1% (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.

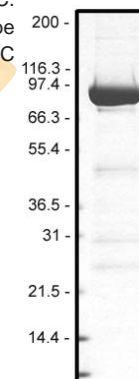
The protein is stable for 6 months if stored at -70°C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 6 months

Purity

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

Note: Breakdown products of recombinant gelsolin are present at 50-25 kDa as determined by immunoblot analysis.

Figure 1. Gelsolin Protein Purity Determination. A 10 µg sample of recombinant gelsolin 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

Gelsolin belongs to a class of actin severing and capping proteins called class I F-actin capping proteins (2). Each of these class I proteins contains a series of conserved 125-150 amino acid repeat motifs. Gelsolin is characterized by the presence of six repeated motifs, three of which are actin binding domains (see Figure 3). Gelsolin exerts a powerful regulatory role on actin filament length and its activity can be modulated by Ca^{2+} levels (3), pH (4), Polyphosphoinositides (5) and post translational modification (6).

The biological activity of recombinant gelsolin can be determined from an F-actin severing assay (see Figure 2). F-actin is incubated with gelsolin and then separated into soluble and insoluble fractions by high speed centrifugation. The amount of actin in the supernatant (G-actin) versus the pellet (F-actin) is compared to a control reaction incubated without gelsolin. Stringent quality control ensures that recombinant gelsolin (2 μg) can solubilize approx. 80% of F-actin (5 μg) in 10 min *in vitro*.

Reagents

- 1) Plasma gelsolin protein (20 μg , Cat. # HPG6)
- 2) Pre-formed F-actin filaments (Cat. # AKF99)
- 3) Reaction buffer (50 mM Tris pH 7.5, 0.1 mM CaCl_2 , 0.1 MgCl_2 , 30 mM NaCl, 1 mM DTT)

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) Protein electrophoresis apparatus

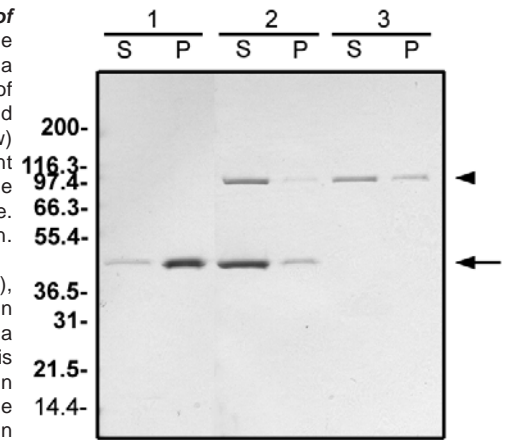
Method

- 1) Dilute the recombinant gelsolin protein to 0.5 mg/ml in cold reaction buffer. Keep on ice.
- 2) **Optional.** Centrifuge the gelsolin protein at 100,000 x g at room temperature for 10 min to pellet any denatured protein; pipette the plasma gelsolin supernatant into a labeled centrifuge tube on ice.
- 3) Resuspend the pre-formed F-actin to 0.4 mg/ml with 2.4 ml of room temperature water.
- 4) Add 45 μl of reaction buffer and 5 μl of F-actin to an ultracentrifuge tube. This is the "actin only" control reaction.
- 5) Add 48 μl of reaction buffer and 2 μl of plasma gelsolin to an ultracentrifuge tube. This is the "gelsolin only" control reaction.
- 6) Add 43 μl of reaction buffer, 5 μl of F-actin and 2 μl of gelsolin to two ultracentrifuge tubes. These are duplicate "actin severing" reactions.
- 7) Incubate all tubes at room temperature for 10 minutes.
- 8) Centrifuge the tubes at 100,000 x g for 30 min to pellet the F-actin.
- 9) Remove the top 95% of the supernatant of each ultracentrifuge tube to a labeled microfuge tube. Avoid touching the bottom of the tube or disturbing the pellet material. Add 10 μl of 5x SDS-sample buffer to each tube.
- 10) Resuspend the pellet fraction (F-actin) in each ultracentrifuge tube with 50 μl of water and 10 μl of 5x SDS- sample buffer.
- 11) Load 30-40 μl of the supernatant and pellet samples on an SDS-gel and run until the dye front is 0.5 cm from then end of the gel. Stain the gel with Coomassie Blue.
- 12) The results of a typical F-actin severing assay is shown in Figure 2.

Figure 2. F-actin Severing Activity of Recombinant Plasma Gelsolin.

The severing activity of recombinant plasma gelsolin was assessed by SDS-PAGE of proportionally loaded supernatant (S) and pellet (P) fractions from F-actin (arrow) incubated with and without recombinant plasma gelsolin (arrowhead) according to the assay method. Sample 1, F-actin alone. Sample 2, F-actin and plasma gelsolin. Sample 3. Plasma gelsolin alone.

In the absence of plasma gelsolin (sample 1), approx. 80% of F-actin (43 kDa) is found in pellet fraction. Upon incubation with plasma gelsolin (sample 2), 80% of the F-actin is severed into soluble G-actin and is found in the supernatant fraction. Note: some recombinant plasma gelsolin can be found in the pellet fraction after centrifugation in all samples. Mark12 molecular weigh markers are from Invitrogen.



Unit Definition

1 unit of activity severs 1 μg of F-actin in a 50 μl volume in 10 min at 24°C in reaction buffer.

Product Uses

- * Positive control for the studying the activity of F-actin severing and capping proteins
- * Investigation of the the effect of actin binding proteins (ABP's) on actin dynamics
- * Study of F-actin gels in bronchial and blood research

References

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