V. 3.0

Plasma Gelsolin
(Human recombinant
Cat. # HPG6

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

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 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 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 $MgCl_2$, 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 >85% pure (see Figure 1).
Note: Breakdown products of recombinant gelsolin are present at 50-25 kDa as determined by immunoblot analysis.

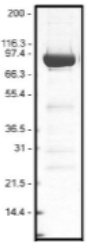

Figure 1. Gelsolin Protein Purity Determination. A 10 µg sample of recombinant gelsolin was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision RedTM Protein Assay Reagent (Cat. # ADV02). Mark12TM molecular weight 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 monomer and polymer 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 can solubilize F-actin *in vitro* (see data below).

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 46 µl of reaction buffer and 4 µl of plasma gelsolin to an ultracentrifuge tube. This is the “gelsolin only” control reaction.
6. Add 41 µl of reaction buffer, 5 µl of F-actin and 4 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 with the pipette tip. Add 10 µl of 5x SDS-sample buffer to each tube containing supernatant material.
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.

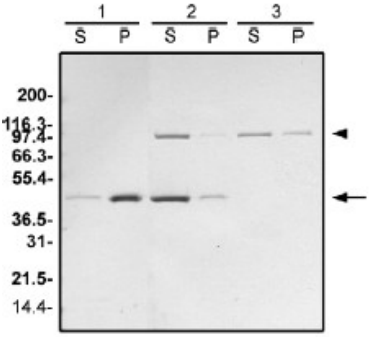

Figure 2. F-actin Severing Activity of Recombinant Plasma Gelsolin. The severing activity of recombinant plasma gelsolin was assessed by SDS-PAGE of proportionally loaded supernatant (S) and pellet (P) fractions from F-actin (arrow) incubated with and without recombinant plasma gelsolin (arrowhead) according to the assay method. Sample 1, F-actin alone. Sample 2, F-actin and gelsolin. Sample 3. Gelsolin alone.
In the absence of gelsolin (sample 1), > 75% of F-actin (43 kDa) is found in pellet fraction. Upon incubation with gelsolin (sample 2), >70% of the F-actin is found in the soluble G-actin form 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.

Product Uses

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

References

1. Kwiatkowski, D.J., et al. 1986. Nature. 323: 455-458.
2. Hartwig, J., and Kwiatkowski, D. 1991. Curr. Opin. in Cell Biol. 3:87-97.
3. Janmey, P. A., et al. 1985. Biochem. 24:3714-3723.
4. Lamb, J., et al. 1993. J. Biol. Chem. 268:8999-9004.
5. Janmey, P. A., and Stossel, T.P. 1987. Nature. 325:362-364.
6. Huckriede, A., et al. 1990. Cell Motil. and Cytoskel. 16:229-238.

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