

Vimentin Protein
(Syrian Hamster)
Cat. # V01

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

Material

Recombinant Syrian hamster vimentin (approximately 99.6% homologous to human vimentin) has been produced and purified from a bacterial expression system. The recombinant protein is untagged and has an approximate molecular weight of 54 kDa. Vimentin can polymerize into vimentin intermediate filaments and can be found in many cells of mesenchymal origin including fibroblasts, endothelial cells and white blood cells. Vimentin protein is supplied in monomer form 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 10 µl of Milli-Q water. The protein will be in the following buffer: 5 mM PIPES 7.0, 1 mM 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. Vimentin protein was determined to be 80% pure (see Figure 1)

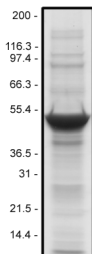


Figure 1. Vimentin Protein Purity Determination. A 10 µg sample of vimentin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of vimentin can be determined by its ability to polymerize into intermediate filaments in vitro and separated from unpolymerized vimentin in a spin down assay. Stringent quality control ensures that approximately 90% of the vimentin protein can polymerize in this assay.

Reagents

1. Vimentin protein (50 µg, Cat. # V01)
2. Vimentin Subunit Buffer (5 mM PIPES pH 7.0, 1.0 mM DTT)
3. 20x Vimentin Polymerization Buffer (100 mM PIPES pH 7.0, 20 mM DTT, 3.4 M NaCl)
4. Precision Red Protein Assay Reagent (Cat. # ADV02)

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. Spectrophotometer capable of measuring absorbance at 600 nm.

Method

1. Dilute vimentin protein (Cat. # V01) to 0.5 mg/ml with cold Vimentin Subunit Buffer.
2. Incubate the protein on ice for 30 min.
3. Centrifuge the protein in a 4°C microfuge at 14k rpm for 15 min.
4. Transfer the supernatant to a new microfuge tube and determine the total protein concentration with the Precision Red Protein Assay Reagent.
5. Pipet 100 µl of the vimentin solution to an airfuge tube.
6. Add 5 µl (1/20th the volume) of Vimentin Polymerization Buffer to the tube and mix well.
7. Incubate at 35°C for 30 min.
8. Centrifuge at 100,000 x g for 1 h to pellet the polymerized vimentin.
9. Remove the top 90% of the supernatant to a clean microfuge tube.
10. Determine the concentration of the protein in the supernatant (unpolymerized vimentin) with the Precision Red Protein Assay Reagent. This protein concentration is used to determine the efficiency with which vimentin polymerized and pelleted during centrifugation.
11. Samples of the supernatant and pellet fractions can be resuspended with Laemmli sample buffer and separated by SDS-PAGE.
12. A typical polymerization assay gel is shown in Figure 2.

NOTE: The extent of vimentin polymerization is dependent on the protein concentration. Filament formation is found to increase linearly with total protein concentration. The critical concentration of vimentin is defined as 10% of the total vimentin concentration.

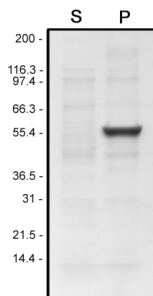


Figure 2. Vimentin Polymerization Assay. The ability of vimentin (54 kDa) to polymerize into intermediate filaments was determined according to the biological assay method. Samples of the supernatant and pellet fractions were collected after polymerization and centrifugation and electrophoresed on an SDS gel. In the presence of 150 mM NaCl >95% of the vimentin protein polymerized into filaments that were pelleted by centrifugation (S, supernatant fraction, P, pellet fraction, arrowhead). Mark12 molecular weight markers are from Invitrogen.

Product Uses

- Investigating vimentin filament dynamics
- Identification of vimentin associated proteins
- Discovery of intermediate filament associated drugs
- Protein kinase substrate
- Protein standard for Western blots

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.