

Material

The VCA (Verprolin, Cofilin, Acidic) domain of human WASP protein has been expressed in a bacterial expression system as a GST-tagged fusion protein. The protein has been purified by glutathione affinity chromatography and is supplied lyophilized in 500 µg aliquots. When reconstituted with distilled water, the complex is at 1 mg/ml in the following buffer: 20 mM Tris pH 7.5, 25 mM KCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.2% dextran and 2% sucrose. The molecular weight of the VCA-GST domain is approximately 43 kDa.

Storage and Reconstitution

Store at 4°C desiccated or at -70°C frozen. Store lyophilized product desiccated (<10% humidity) at 4°C. Resuspend the protein complex to 1 mg/ml with cold Milli-Q water. The protein should then be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at -70°C where it is stable for 6 months. Avoid multiple freeze-thaw cycles. Further dilution of VCA-GST should be made in the following buffer: 20 mM Tris pH 7.5, 20mM NaCl.

Purity

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

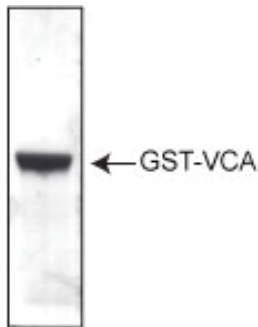


Figure 1. WASP-VCA Domain Protein Purity Determination. A 20 µg sample of VCG03 was run on an SDS-PAGE gel and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat# ADV02).

Biological Activity Assay

The Arp2/3 complex is able to induce the branched polymerization of actin filaments *in vitro* at a molar ratio of 1:200 (Arp2/3:actin). This stimulation is observable in an *in vitro* polymerization assay (described below), however, the stimulation from Arp2/3 alone is very low under these conditions. In the presence of VCA-GST protein the nucleating activity of Arp2/3 is greatly enhanced. The *in vitro* polymerization assay is described below:

Reagents

1. Arp2/3 protein complex (Cat. # RP01)
2. VCA domain-GST fusion (Cat # VCG03). This is the COOH-terminal domain of N-WASP containing a Verprolin-homology region, a Cofilin homology region and an Acidic terminal segment.
3. Pyrene labeled actin (Cat. # AP05)
4. Polymerization buffer **1.5x stock**: 7.5 mM Tris pH 7.5, 75 mM KCl, 3 mM MgCl₂, 1.5 mM EGTA, 0.15 mM CaCl, 0.75 mM DTT, 0.3 mM ATP [add fresh from a 100 mM stock pH 7.0, immediately prior to use].
5. General Actin Buffer (Cat. # BSA01): 10 mM Tris pH 7.5, 0.2 mM CaCl₂, 0.2 mM ATP, 1 mM DTT.

Equipment

1. Fluorescence spectrophotometer with an excitation wavelength of 360 +/- 20 nm and an emission wavelength of 405 +/- 10 nm or 420 +/- 20 nm. Cytoskeleton recommends the SPECTRAFluorPlus from TECAN Austria GmbH or Gemini from Molecular Devices Inc.
2. Black polystyrene 96 well assay plate (Costar, Cat. # 3915).

Method

1. Resuspend and dilute pyrene labeled muscle actin (Cat. # AP05) to 0.45 mg/ml (10.4 µM) with General Actin Buffer (Cat. # BSA01) supplemented with 0.2 mM ATP and 1 mM DTT. Leave on ice for 1 h to depolymerize actin oligomers.
2. Centrifuge the actin at 14,000 rpm at 4°C for 30 min to remove residual nucleating centers.
3. Pipette the top 80% of the supernatant into a new microfuge tube on ice.
4. Dilute the Arp2/3 complex (Cat. # RP01) to 0.3 mg/ml in G-buffer. Keep on ice.
5. Resuspend one tube of VCA domain protein (Cat. # VCG03) to 1 mg/ml by adding 500 µl of Milli-Q water. Pipet up and down slowly to resuspend the pellet. Keep on ice.
6. Just before use, dilute the pyrene-labeled actin to 0.1 mg/ml in ice-cold General Actin Buffer.

7. Add the following components to the 96 well assay plate:

Well	Poly. buffer (μ l)	RPD1 (μ l)	VCA domain (μ l)
A1	200	0	0
B1	200	0	0
C1	200	0	5
D1	200	0	5
E1	200	2	0
F1	200	2	0
G1	200	2	5
H1	200	2	5

8. Using a multi-channel pipet, add 100 μ l of diluted pyrene-labeled actin to wells A1-H1 of the assay plate. **Note:** Do not introduce air bubbles into the wells.
9. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 1 h.
10. In the assay described above, pyrene actin is present at a final concentration of 0.8 μ M, Arp2/3 complex at 10 nM and VCA domain at 400 nM.
11. Results for a typical actin polymerization assay is shown in Figure 2.

Product Uses

- Stimulating Arp2/3 Complex activity
- Isolating Arp2/3 Complex from cell or tissue extract

References

1. Pollard, T.D. et al. 2000. *Ann. Rev. Biophys. Biomol. Struct.* 29: 545-576.

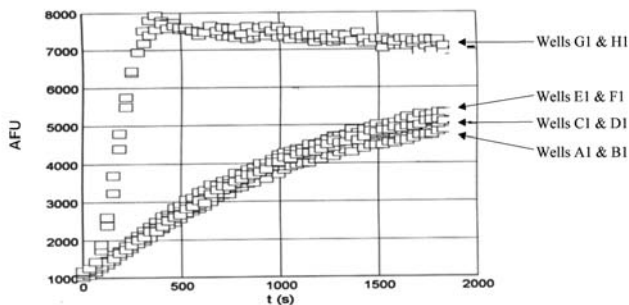


Figure 2. Actin Polymerization Assay with Arp2/3 and VCA domain proteins. Actin polymerization was carried out as described in the method; all reactions contain pyrene labeled actin. Reactions containing Arp2/3 or VCA in addition to actin show little or no enhancement of actin nucleation. In the presence of the VCA domain however, Arp2/3 results in an enhancement of actin nucleation (Note the steep nucleation phase of polymerization). Actin polymerization is measured in arbitrary fluorescent units over time.