V. 2.0

WASP-VCA Domain-GST Protein

Cat. # VCG03
Lot: Amount: 1 x 500 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material
The VCA (Verprolin, Cofilin, Acidic) domain (aa 392-505) 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 500 µl of distilled water, the complex is at 1 mg/ml in the following buffer: 20 mM Tris pH 7.5, 25 mM KCl, 1 mM $MgCl_2$, 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 lyophilized product desiccated (<10% humidity) at 4°C. Resuspend the protein complex to 1 mg/ml with 500 µl of 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, 20 mM 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 90% 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. # RP01P)
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 $MgCl_2$, 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 $CaCl_2$, 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 SPECTRAFluor Plus from TECAN Austria GmbH or Gemini from Molecular Devices Inc.
2. Black polystrene 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. # RP01P) 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:

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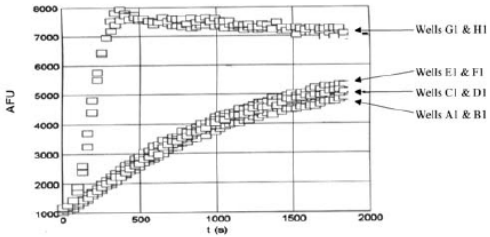
8. Using a multi-channel pipet, add 100 µl of diluted pyrene-labeled actin to wells A1 to H1 of the assay plate. **Note:** Do not introduce air bubbles into the wells.

Well	Poly. Buffer (µl)	RP01P (µl)	VCA domain (µl)
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

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 µM, 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.

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.

References

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

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