V. 1.1

Vav2 Protein: DH domain (αα 189-374).
(Human recombinant, 6xHis tagged)
Cat. # CS-GE06
Lot 011 Amount 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background

Vav2 protein is a guanine exchange factor with selectivity for Rac1, which mediates Rac1 activation under a variety of conditions and has been associated with gastric tumors as well as cellular volume control and neurite extension (Refs. 1,2,3).

Material

The DH domain of human Vav2 protein has been produced in a bacterial expression system. It contains a 6xHis tagged at its amino terminus for purification purposes. The accession number is NM_001134398.1. The molecular weight of GE06 is approximately 25 kDa. The Vav2 DH protein is supplied as a white lyophilized powder. Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. Vav2 DH protein was determined to be approximately 80% pure. (see Figure 1).

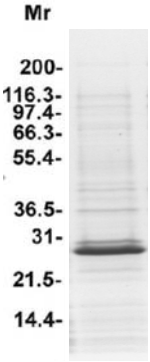


Figure 1. Purity Determination.
Legend: A 5 µg sample of recombinant Vav2 DH (molecular weight approx. 25 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Life Technologies Inc.

Storage and Reconstitution

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with nanopure water (20 µl water per 100 µg protein). When reconstituted, the protein will be in the following buffer: 20 mM Tris pH 7.5, 50 mM NaCl, 5% sucrose and 1% dextran. In order to maintain high biological activity of the protein it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for six months if stored at -70°C. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year. Further dilutions should be made in Dilution Buffer (not supplied: 20 mM Tris pH 7.5, 50 mM NaCl, 1 mM $MgCl_2$, and 100 µg/ml BSA).

Biological Activity Assay

The biological activity of Vav2 DH can be determined from its ability to catalyze nucleotide exchange on Rac1 using the nucleotide exchange assay of Bodipy-GDP for excess GDP or GTP. Rac1 protein is pre-loaded with Bodipy-FL-GDP by adding excess EDTA e.g. 0.7 mmol EDTA per mmol Mg2+ ions present in the reaction. This sub-stock solution is then used in a dissociation assay format which indicates competition for exchange site with unlabeled nucleotide. The reaction is monitored by fluorescence measurement at 485nm Ex / 535nm Em. Stringent quality control ensures that the exchange rate of Bodipy-GTP or mant-GTP is enhanced at least five fold in the presence of 0.8 µM Vav2 DH.

Reagents, Materials and Equipment

1. Vav2 DH protein (Cat. # CS-GE06)
2. Rac1 protein (Cat. # RC01)
3. Exchange buffer 2 (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT, 2 mM EDTA, 100 µg/ml BSA, and 0.75 µM Bodipy-GDP), note - make fresh.
4. 50 mM $MgCl_2$ in 20 mM Tris-HCl pH 7.5, 50 mM NaCl.
5. 5 mM GTP in 20 mM Tris-HCl pH 7.5, 50 mM NaCl..
6. 96-well plate fluorescence spectrophotometer.
7. Fluorescence half area low-binding black 96-well plate (Corning Cat. # 3686).

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

Method

1. Place Vav2 vial on ice and dilute to 0.20 µg/µl (8 µM) with ice cold Exchange Buffer.

2. Dilute Rac1 (Cat# RC01) to 1.25 µg/µl (50 µM) with ice cold Exchange Buffer..

3. Add the following components together in a fresh 15 ml Falcon tube and mix well by pipetting or gentle vortex:

Component	per well
Exchange Buffer	75 µl
50 µM Rac1	5 µl
8 µM Vav2	10 µl

Note: For a total mixture volume, multiply the volume of reagents per well by the number of wells in the experiment, plus add 20% volume for pipetting losses.

5. Incubate for 20 min at room temperature (RT).

6. Lock in the nucleotide by adding 10 µl (per well) of 50 mM $MgCl_2$.

7. Set up the fluorimeter with Excitation wavelength at 485 nm +/-20 nm and emission wavelength at 535 nm +/- 20 nm at RT.

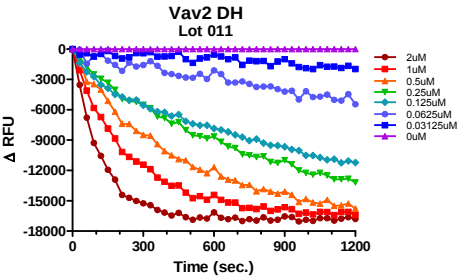
8. Aliquot the pre-loaded mixture to the assigned wells and place the plate in the fluorimeter.

9. After 5 cycles (150 seconds), place the program on Hold or Pause, and remove the plate.

10. Pipette 10 µl of a) 5 mM GTP solution, b) a small compound, c) a test protein, d) 4 mM EDTA (+ve exchange control) or e) Dilution Buffer (negative control) in respective wells and immediately pipette up and down twice and resume reading for 20 minutes.

11. Save the readings after the kinetic protocols are finished. The exchange rate can be calculated by reducing the data to max slope (using 12 pts) or Vmax with the software that accompanies the plate reader. The exchange curve can be achieved by export to Microsoft Excel.

Figure 2. Vav2 catalyzed Bodipy-GDP dissociation assay.



Legend: Rac1 protein (Cat. # RC01) (2.5 µM) was pre-loaded with Bodipy-FL-GDP using EDTA for exchange. The nucleotide was locked in place with excess Mg^{2+}. Vav2 at different concentrations as shown or Dilution Buffer (purple) was pipetted into wells of a black 384-well low volume plate. At time zero, 500 µM GTP was pipetted in to the wells and the reactions were monitored for 20 min by reading every 30 sec..

References

1. Tan B.B. et al. 2017. Up-regulated Vav2 in gastric cancer tissues promotes tumor invasion and metastasis. *Tumour Biol.* **39**(5):1010428317698392.
2. Salin-Cantegrel A. et al 2013. Potassium-chloride cotransporter 3 interacts with Vav2 to synchronize the cell volume decrease response with cell protrusion dynamics. *PLoS One*. **8**(5):e65294.
3. Moon M.S. et al. 2010. Balanced Vav2 GEF activity regulates neurite outgrowth and branching in vitro and in vivo. *Mol Cell Neurosci*. **44**(2):118-28.

Product Uses

- Study of Vav2 interacting proteins, such as Rac1.
- Identification of Vav2 interacting proteins.
- Drug discovery tool for Vav2/Rac1 pathway inhibitors.
- Positive control for Western blots.

Product Citations/Related Products

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