

Active Recombinant Human VAV1 Protein, Y174D Mutant, His tagged

Cat. No. VAV1-23H Lot. No. (See product label)

SPECIFICATION

Product Overview The DHPHC1 domain of human Vav1 protein has been produced in a bacterial expression system. It contains a mutated amino-acid that mimics tyrosine phosphorylation which is required for interaction with Rac1 (Y174D mutant). It is also 6xHis tagged at its amino terminus for purification purposes.

Species Human

Source Bacteria

ProteinLength 168-522 aa

Description This gene is a member of the VAV gene family. The VAV proteins are guanine nucleotide exchange factors (GEFs) for Rho family GTPases that activate pathways leading to actin cytoskeletal rearrangements and transcriptional alterations. The encoded protein is important in hematopoiesis, playing a role in T-cell and B-cell development and activation. The encoded protein has been identified as the specific binding partner of Nef proteins from HIV-1. Coexpression and binding of these partners initiates profound morphological changes, cytoskeletal rearrangements and the JNK/SAPK signaling cascade, leading to increased levels of viral transcription and replication. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.

Form White lyophilized powder

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

Bio-activity

The biological activity of Vav1 DHPHC1 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 the 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-GDP or mant-GDP is enhanced at least five fold in the presence of 0.8 μM Vav1.

Molecular Mass 41 kDa

Purity

Protein purity is determined by scanning densitometry of Coomassie blue stained protein on a 4-20% polyacrylamide gradient gel. The Vav1 protein was determined to be > 80% pure.

Applications

1. Study inhibitors of Vav1 GTP/GDP exchange activity
2. Identification of Vav1 DHPHC1 domain binding proteins
3. Study of Vav1 GEF activity with different GTPases

Usage

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/mL with ice cold 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, 1 mM MgCl₂, 5% (v/v) sucrose and 1% (v/v) 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 centigrade. The protein is stable for six months if stored at -70 centigrade. The protein must not be exposed to repeated freeze-thaw cycles. Further Vav1 dilutions should be made in 20 mM Tris pH 7.5, 50 mM NaCl, 1 mM

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MgCl₂ (not supplied).

Storage

The lyophilized protein is stable at 4 centigrade desiccated (<10% humidity) for one year.

References

1. Zhu X. et al. 2017. Vav1 expression is increased in esophageal squamous cell carcinoma and indicates poor prognosis. *Biochem Biophys Res Commun.*, 486(2):571-576.
2. Guerreiro-Cacais A.O. et al. 2017. VAV1 regulates experimental autoimmune arthritis and is associated with anti-CCP negative rheumatoid arthritis. *Genes Immun.* 18(1):48-56.
3. Razanadrakoto L. et al. 2015. Mutation of Vav1 adaptor region reveals a new oncogenic activation. *Oncotarget.* 10; 6(4):2524-37.

GENE INFORMATION

Gene Name

VAV1 vav guanine nucleotide exchange factor 1 [Homo sapiens (human)]

Official Symbol

VAV1

Synonyms

VAV1; vav guanine nucleotide exchange factor 1; VAV; proto-oncogene vav; vav 1 guanine nucleotide exchange factor; vav 1 oncogene

Gene ID

7409

mRNA Refseq

NM_005428

Protein Refseq


NP_005419

MIM

164875

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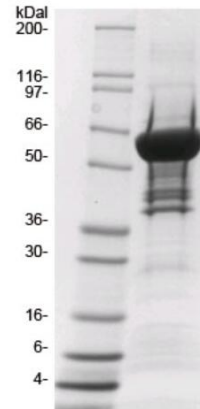
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UniProt ID

P15498

Purity



Legend: A 5 µg sample of recombinant Vav1 DHPHC1 (molecular weight approx. 41 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. Mark12 molecular weight markers are from Life Technologies Inc.

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