Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active VCPIP1 [25 – 561]

Enzyme description:- VCPIP1 [25 - 561]

Clone number:- DU 44386

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 86, 538.24 daltons
Average Mass 86, 594.01 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.57

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35

Storage temperature:- -70 °C

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Clone Data Sheet

VCPIP1 [25 - 561]

Protein	VCPIP1[25 - 561]
Clone number	DU 44386
Species	Human
Accession number	Q96JH7
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFM**SSLASAAASG** **GLLKRRDRRILSGSCPDPKCQARLFFPASGSVSIECTECGQRHEQQQLL** **GVEEVTDPDVVLHNLLRNALLGVTGAPKKNTELVKVMGLSNYHCKLLSP** **ILARYGMDKQTGRAKLLRDMNQGELFDCALLGDRAFLIEPEHVNTVGYG** **KDRSGSLLYLHDTLEDIKRANKSQECLIPVHVDGDGHCLVHAVSRALVG** **RELFWHALRENLKQHFQQHLARYQALFHDFIDAAEWEDIINECDPLFVP** **PEGVPLGLRNIHIFGLANVLHRPIILLDSLSGMRSSGDYSATFLPGLIP** **AEKCTGKDGHLNKPICIAWSSSGRNHYIPLVGIKGAALPKLPMNLLPKA** **WGVPQDLIKKYIKLEEDGGCVIGGDRSLQDKYLLRLVAAMEEVFMDKHG** **IHPSLVADVHQYFYRRTGVIGVQPEEVTAAAKKAVMDNRLHKCLLCGAL** **SELHVPPEWLAPGGKLYNLAKSTHGQLRTDKNYSFPLNNLVCSYDSVKD** **VLVPDYGMSNLTACNWCHGTSVRKVRGDGSIVYLDGD**
Native sequence	Amino acids S25 – D561 (end residue is S1222) of human VCPIP1. Residue S236 of the fusion protein is equivalent to S25 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Eco*R1 and *Not*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

gaattcatgTCGTCCTTGGCGTCGGCGGCTGCTTCGGGGGGGCTTTTGA
AGCGGAGAGACCGGAGAATCCTTTCCGGGAGCTGCCCGGATCCGAAGTG
TCAGGCGCGTCTATTTTTCCCGGCCTCCGGTTCTGTCAGCATCGAGTGT
ACCGAGTGCGGCCAGCGGCACGAGCAGCAACAGCTGCTGGGGGTTGAGG
AGGTGACCGACCCGGACGTAGTGCTACACAACCTGCTGCGGAACGCGCT
GCTCGGGGTTACGGGGGCACCCAAGAAGAACACGGAACTGGTAAAGGTG
ATGGGCCTTTCCAACTATCACTGCAAATTGTTGTCGCCCATATTAGCTC
GCTATGGAATGGACAAACAGACAGGCCGGGCCAAGCTTCTCCGGGACAT
GAACCAGGGCGAACTGTTCGATTGCGCCTTACTGGGTGACCGCGCCTTC
CTCATAGAACCAGAGCATGTTAACACTGTGGGCTATGGCAAGGACCGCT
CCGGAAGCCTCCTGTATTTGCATGACACTCTGGAGGACATTAAGCGGGC
CAATAAAAGCCAGGAATGTCTCATTCCAGTGCATGTGGACGGGGATGGA
CACTGCTTGGTGCATGCTGTGTCTCGGGCTCTAGTAGGCCGAGAGCTCT
TCTGGCATGCCTTAAGAGAGAATCTTAAACAGCACTTTCAGCAGCACCT
GGCCCGATATCAAGCTCTGTTCCATGACTTCATTGATGCTGCTGAGTGG
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ACATCGTCCTATTATTCTGTTAGATTCCCTCAGTGGCATGAGAAGCTCT
GGTGATTATTCAGCCACCTTTCTACCTGGGCTCATCCCTGCAGAGAAGT
GCACTGGGAAAGATGGTCATTTGAACAAACCAATCTGTATTGCATGGAG
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TTGAACAATTTGGTTTGCTCATATGATTCAGTGAAAGATGTTCTGGTAC
CAGACTATGGAATGAGTAACCTAACAGCTTGTAATTGGTGCCATGGCAC
ATCTGTGCGAAAGGTCAGAGGAGATGGGTCTATTGTGTATTTGGATGGA
GACtagcggccgc