

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-VCPIP1

<u>Enzyme description:-</u>	GST-VCPIP1
<u>Clone number:-</u>	DU25041
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	1.2 mg/L

Calculated molecular mass:-

Monoisotopic	160897 Da
Average Mass	160994 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 6.93

Purity:- 60%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 µM Ub-Rho110-Gly

Division of Signal Transduction Therapy

Clone Data Sheet

GST-VCPIP1

<u>Protein</u>	GST-VCPIP1
<u>Synonyms</u>	VCPIP135
<u>Clone Number</u>	DU25041
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96JH7 DNA: NM_025054.4
<u>Tags</u>	N-terminal GST
<u>Amino acid sequence of expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG GDHPPKSDENLYFOGGSMSQPPPPPPPLPPPPPPPEAPQTPSSLASAAASGGL LKRRDRRILSGSCPDKCOARLFFPASGSVSI ECTECGQRHEQQOLLGVEEVT DPDVVLHNLRLNALLGVTGAPKKNTELVKVMGLSNYHCKLLSPILARYGMDKQ TGRAKLLRDMNQGELFDCALLGDRAFLIEPEHVNTVGYGKDRSGSLLYLHDTL EDIKRANKSQECLIPVHVDGDGHCLVHAVSRALVGRELFWHALRENLKQHFQO HLARYQALFHDFIDAAEWEDI INECDPLFVPPPEGVPLGLRNIHIFGLANVLR PIILLDSL SGMRS GDYSATFLPGLIPA EKCCTGKDGH LNKPICIAWSSSGRNH YIPLVGIKGAALPKLPMNLLPKAWGVPQDLIKKYIKLEEDGGCVIGGDRSLQD KYLLRLVAAMEEVFMDKHGIHPSLVADVHQYFYRRTGVIGVQPEEVTAAAKKA VMDNRLHKCLLCGALSELHVPPEWLAPGGKLYNLAKSTHGQLRTDKNYSFPLN NLVCSYDSVKDVLVPDYGMSNL TACNWCHGTSVRKVRGDGSIVYLDGDR TNSR STGGKCGCGFKHFWDGKEYDNLPEAFPITLEWGGRVVRET VYWFQYESDSSLN SNVYDVAMKLVTKHFPGEFGSEILVQKVVHTILHQ TAKKNPDDYTPVNI DGAH AQRVGDVQGOESESQLP TKIILTGQKTKTLHKEELNMSKTERTIQONITEQAS VMQKRKTEKLKQEQKQPRTVSPSTIRDGPSSAPATPTKAPYSPTTSKEKKIR ITTNDGRQSMVTLKSSTTFFELQESIAREFNIPPYLQCI RYGFPPKELMPPQA GMEKEPVPLQHGDRITIEILKS KAEGGQSAAAHSAHTVKQEDIAVTGKLS SKE LQEQA EKEMYS LCLLATLMGEDVWSYAKGLPHMFOGGVFYSIMKKTMG MADG KHCTFPHLPGKTFVYNASEDRLELCVDAAGHFPIGPDVEDLVKEAVSQVRAEA TTRSRESSPSHGLLKLGS GGVVKKKSEQLHNVTAFQGGKHS LGTASGNPHLDP RARETSVVRKHNTGTDFSNSSTKTEPSVFTASSNSELIRIAPGVV TMRDGRQ LDPDLVEAQRKKLQEMVSSIQASMDRHLRDQSTEQSPSDLPQRKTEVVSSSAK SGSLQ TGLPESFPLTGGTENLNTETTDGCVADALGA AFATRSKAQRGNSVEEL EEMDSQDAEMTNTTEPMDHS</p>
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	site underlined
<u>Cloning sites</u>	BglII/Not1

DNA sequence of insert

AGATCTATGTCTCAGCCGCCGCCGCCGCCCTCCGTTGCCGCCGCCACCTCC
TCCCCCTGAGGCTCCACAGACTCCGTCGTCCTTGGCGTCGGCGGCTGCTTCGG
GGGGGCTTTTGAAGCGGAGAGACCGGAGAATCCTTTCCGGGAGCTGCCCGGAT
CCGAAGTGTCAAGCGCGTCTATTTTTCCCGGCCTCCGGTCTGTGTCAGCATCGA
GTGTACCGAGTGCGGCCAGCGGCACGAGCAGCAACAGCTGCTGGGGGTTGAGG
AGGTGACCGACCCGGACGTAGTGCTACACAACCTGCTGCGGAACGCGCTGCTC
GGGTTACGGGGGCACCCAAGAAGAACACGGAACCTGGTAAAGGTGATGGGCCT
TTCCAACATCACTGCAAATGTTGTGCGCCATATTAGCTCGCTATGGAATGG
ACAAACAGACAGGCCGGGCCAAGCTTCTCCGGGACATGAACCAGGGCGAACTG
TTCGATTGCGCCTTACTGGGTGACCGCGCCTTCCATAGAACAGAGCATGT
TAACACTGTGGGCTATGGCAAGGACCGCTCCGGAAGCCTCCTGTATTTGCATG
ACACTCTGGAGGACATTAAGCGGGCCAATAAAAGCCAGGAATGTCTCATTTCCA
GTGCATGTGGACGGGGATGGACACTGCTTGGTGCATGCTGTGTCTCGGGCTCT
AGTAGGCCGAGAGCTCTTCTGGCATGCCTTAAGAGAGAATCTTAAACAGCACT
TTCAGCAGCACCTGGCCCCGATATCAAGCTCTGTTCCATGACTTCATTGATGCT
GCTGAGTGGGAGGACATTATCAATGAGTGTGACCCTCTGTTTGTACCACCTGA
GGGTGTTCCCTTGGGCCTGAGGAATATCCACATATTTGGTCTTGCCAATGTGC
TACATCGTCCTATTATTCTGTTAGATTCCCTCAGTGGCATGAGAAGCTCTGGT
GATTATTCAGCCACCTTTCTACCTGGGCTCATCCCTGCAGAGAAGTGCCTGG
GAAAGATGGTCATTTGAACAAACCAATCTGTATTGCATGGAGCAGCTCCGGTA
GAAACCATTATATCCCCTTGGTAGGCATAAAAGGGGCTGCTTTGCCAAACTG
CCTATGAATTTGCTTCCCTAAAGCATGGGGTGTGCCTCAGGACCTTATTA
GTACATAAAACTTGAAGAGGATGGTGGTTGTGTTATTGGAGGTGACAGAAGTT
TGCAAGATAAAATACTTACTTAGGCTTGTGCTGCTATGGAAGAAGTCTTTATG
GACAAACATGGTATCCATCCTAGTTTGGTTGCTGATGTCCATCAGTATTTCTA
CAGAAGGACTGGAGTGATAGGAGTTCAGCCTGAGGAAGTCACAGCAGCTGCTA
AAAAAGCAGTAATGGATAATCGCCTTACAAATGTTTGTCTGTGGTGCCTT
TCTGAACCTTCATGTTCCCTCCAGAGTGGTTGGCTCCTGGAGGGAAATTGTATAA
CCTGGCAAAAAGTACTCATGGACAGCTGAGGACTGACAAAAATTACAGCTTTC
CCTTGAACAATTTGGTTTGTCTCATATGATTCAGTGAAAAGATGTTCTGGTACCA
GACTATGGAATGAGTAACCTAACAGCTTGTAATTGGTGCCATGGCACATCTGT
GCGAAAGGTCAGAGGAGATGGGTCTATTGTGTATTTGGATGGAGACAGAACTA
ATTCTAGGTCCACTGGTGGCAAATGTGGTTGTGGATTCAAACACTTTTGGGAT
GGTAAGGAGTATGACAATCTACCAGAAGCTTTCCTTACTTTAGAAATGGGG
TGGAAGAGTGGTCAGAGAAAACAGTATATTGGTTCCAGTATGAAAGTGATTCAT
CTTTGAATAGTAATGTTTACGATGTTGCAATGAAACTTGTTACCAAGCACTTT
CCAGGTGAATTTGGGAGTGAAATCCTAGTTCAGAAAGTTGTCCACACTATATT
GCATCAGACTGCCAAAAAGAAATCCCAGATGATTATACTCCTGTAAATATAGATG
GTGCTCACGCCCAAAGAGTTGGAGATGTTCAAGGACAAAGAATCAGAGTCTCAG
CTCCCAACTAAAATTATTCTTACTGGACAGAAAACAAAAACTTTGCACAAGGA
GGAGTTAAACATGAGTAAAACCTGAAAGAACTATTCAACAGAATATTACGGAAC
AGGCTTCTGTAATGCAGAAAACGAAAACAGAGAAGTTAAAACAAGAACAAAA
GGGCAACCCAGGACTGTTTCTCCAGTACCATTTCGTGATGGTCCATCCTCTGC
ACCTGCTACACCTACCAAGGCTCCCTATTACCGACAACCTTCTAAGGAGAAGA
AGATCCGAATCACAACATAATGATGGACGACAGTCCATGGTTACCCTTAAGTCT
TCAACAACCTTTTTTGAACCTCAGGAAAGTATAGCCAGAGAATTCAACATTCC
TCCATATTTACAGTGTATTTCGATACGGGTTTCCCTCCTAAAGAGTTAATGCCAC
CACAGGCAGGAATGGAAAAGGAACCAAGTTCCTTTACAGCATGGCGACAGAATT
ACAATAGAAATTCATAAAAAGTAAAGCTGAAGGTGGTCACTCTGCTGCAGCACA
CTCAGCCACACTGTGAAACAAGAAGATATTGCTGTTACTGGTAAACTGTCAT
CTAAGGAACCTCAGGAGCAAGCTGAAAAAGAAATGTACTCCTTGTGTCTTTTA
GCAACATTAATGGGAGAAGATGTATGGTCTTATGCAAAGGACTTCCCTCATAT
GTTTCAGCAGGGTGGTGTATTCTACAGTATTATGAAGAAAACCATGGGTATGG
CTGATGGCAAGCATTGTACTTTCCACATCTGCCTGGCAAAACCTTTGTCTAT
AATGCTTCTGAAGATAGACTGGAATTGTGTGTGGATGCTGCAGGACATTTCCC
CATTGGTCCCTGATGTTGAAGATTTAGTTAAAGAGGCTGTAAGTCAGGTTCGAG

CAGAGGCTACTACAAGAAGTAGGGAATCAAGTCCCTCACATGGGCTATTAAAA
CTAGGTAGTGGTGGAGTAGTGAAAAAGAAATCTGAGCAACTTCATAACGTAAC
TGCCTTTCAGGGAAAAGGGCATTCTTTAGGAACTGCATCTGGTAACCCACACC
TTGATCCAAGAGCTAGGGAAACTTCAGTTGTAAGAAAGCATAATACAGGGACA
GACTTTAGTAATAGTTCCACTAAAACAGAGCCTTCTGTATTCACAGCTTCTTC
TAGTAATAGTGAGCTTATTCGAATAGCTCCTGGAGTAGTAACAATGAGAGACG
GCAGGCAGCTTGATCCTGATTTGGTTGAGGCCAGCGAAAAAAATTGCAGGAA
ATGGTTTCTTCTATTTCAGGCTTCAATGGACAGGCACCTTCGGGATCAAAGTAC
AGAGCAGTCACCATCTGATCTTCCTCAAAGGAAAACAGAAGTTGTGAGTTCTT
CTGCAAAGTCTGGGAGTCTTCAGACTGGTTTGCCTGAATCTTTTCTTTAACT
GGTGGTACTGAAAATTTGAATACAGAAACAACCTGATGGCTGTGTAGCAGATGC
ACTGGGAGCAGCCTTTGCCACAAGGTCAAAGCACAAAGGGGAAATTCCTGG
AGGAGCTTGAAGAGATGGATAGTCAAGATGCTGAGATGACTAACACAACCTGAG
CCAATGGATCACTCTTGAGCGGCCGC