

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-VCPIP1

<u>Enzyme description:-</u>	His-VCPIP1
<u>Clone number:-</u>	DU25040
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal His
<u>Purification method:-</u>	Ni ⁺⁺ -Sepharose
<u>Expression level:-</u>	1.3 mg/L

Calculated molecular mass:-

Monoisotopic	137170 Da
Average Mass	137252 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 7.18

Purity:- 70%

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

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Clone Data Sheet

His-VCPIP1

Protein His-VCPIP1
Synonyms VCPIP135
Clone Number DU25040
Species Human
Accession Number Protein: Q96JH7 DNA: NM_025054.5
Tags N-terminal His₆-tag

Amino acid sequence of expressed protein **MGSSHHHHHHSSGENLYFQGHMLGSMSQPPPPPPPLPPPPPPPEAPQTPSSLA**
SAAASGGLLKRDRRILSGSCPDKCQARLFFPASGSVSI ECTECCQRHEQQQ
LLGV E E V T D P D V V L H N L L R N A L L G V T G A P K K N T E L V K V M G L S N Y H C K L L S P I L
ARYGMDKQTGRAKLLRDMNQELFDCALLGDRAFLIEPEHVNTVGYGKDRSGS
LLYLHDTLEDIKRANKSQECLIPVHVDGDGHCLVHAVSRALVGRELFWHALRE
NLKQHFQOHLARYQALFHDFIDAAEWEDI INECDPLFV P P E G V P L G L R N I H I F
GLANVLHRPI ILLDSLGMRS S G D Y S A T F L P G L I P A E K C T G K D G H L N K P I C I A
WSSSGRNHYIPLVGIKGAALPKLPMNLLPKAWGVPQDLIKKYIKLEEDGGCVI
GGDRSLQDKYLLRLVAAMEEVFMDKHGIHPSLVADVHQYFYRRTGVI G V Q P E E
VTAAAKKAVMDNRLHKCLLCGALSELHVPPPEWLAPGGKLYNLAKSTHGQLRTD
KNYSFPLNNLVCSYDSVKDVLVPDYGMSNL TACN W C H G T S V R K V R G D G S I V Y L
DGDR T N S R S T G G K C G C G F K H F W D G K E Y D N L P E A F P I T L E W G G R V V R E T V Y W F Q
YESDSSLNSNVYDVAMKLVTKHFPGEFGSEILVQKV V H T I L H Q T A K K N P D D Y T
PVNI D G A H A Q R V G D V Q G Q E S E S Q L P T K I I L T G Q K T K T L H K E E L N M S K T E R T I Q
Q N I T E Q A S V M Q K R K T E K L K Q E Q K G Q P R T V S P S T I R D G P S S A P A T P T K A P Y S P T
TSKEKKIRIT T N D G R Q S M V T L K S S T T F F E L Q E S I A R E F N I P P Y L Q C I R Y G F P P
KELMPPQAGMEKEPVPLQHGDRITIEILKSKAEGGQSAAAHAHTVKQEDIAV
TGKLSSKELQEQAEKEMYSLCLLATLMGEDVWSYAKGLPHMFQGGVFYSIMK
KTMGMADGKHCTFPHLPGKTFVYNASEDRLELCVDAAGHFPIGPDVEDLVKEA
VSQVRAEATTRSRESSPSHGLLKLGGSGGVVKKKSEQLHNVTAFQKGHSLGTA
SGNPHLDPRARETSVVRKHNTGTDFSNSSTKTEPSVFTASSNSELIRIAPGV
VTMRDGRQLDPLVEAQRKKLQEMVSSI QASMDRHLRDQSTEQSPSDLPQRKT
EVVSSSAKSGSLQ T G L P E S F P L T G G T E N L N T E T T D G C V A D A L G A A F A T R S K A Q
RGNSVEELEEMDSQDAEMTNTTEPMDHSZAA

Native sequence in bold
Protease cleavage TEV site underlined
Cloning sites BgIII/BamHI

DNA sequence of insert

AGATCTATGTCTCAGCCGCCGCCGCCGCCCTCCGTTGCCGCCGCCACCTCC
TCCCCCTGAGGCTCCACAGACTCCGTCGTCCTTGGCGTCGGCGGCTGCTTCGG
GGGGGCTTTTGAAGCGGAGAGACCGGAGAATCCTTTCCGGGAGCTGCCCGGAT
CCGAAGTGTCAAGCGCGTCTATTTTTCCCGGCCTCCGGTCTGTGTCAGCATCGA
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AATGCTTCTGAAGATAGACTGGAATTGTGTGTGGATGCTGCAGGACATTTCCC
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CAGAGGCTACTACAAGAAGTAGGGAATCAAGTCCCTCACATGGGCTATTAAAA
CTAGGTAGTGGTGGAGTAGTGAAAAAGAAATCTGAGCAACTTCATAACGTAAC
TGCCTTTCAGGGAAAAGGGCATTCTTTAGGAACTGCATCTGGTAACCCACACC
TTGATCCAAGAGCTAGGGAAACTTCAGTTGTAAGAAAGCATAATACAGGGACA
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