

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Vimentin [2 - 466]

Enzyme description:- Vimentin [2 – 466]

Clone number:- DU 1755

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 80, 294.67 daltons

Average Mass 80, 344.65 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.21

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Vimentin [2 – 466]

<u>Protein</u>	Vimentin [2 – 466]
<u>Clone number</u>	DU 1755
<u>Species</u>	Human
<u>Accession number</u>	NM_003380.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGGPLGSSTRSVSSSSYRRMF GGPGTASRPSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGGVYATR SSAVRLRSSVPGVRLLDQSVDFSLADAINTEFKNTRTNEKVELQELNDR FANYIDKVRFLEQQNKILLAELEQLKGQGSRLGDLYEEEMRELRRQVD QLTNDKARVEVERDNLAEDIMRLREKLQEEMLQREEAENTLQSFQDQD NASLARLDLERKVESLQEEIAFLKKLHEEEIQELQAQIQEQHVQIDVDV SKPDLTAALRDVRQQYESVAAKNLQEAEEWYKSKFADLSEAANRNNDAL RQAKQESTEYRRQVQSLTCEVDALKGTNESLERQMREMEENFAVEAANY QDTIGRLQDEIQNMKEEMARHLREYQDLLNVKMALDIEIATYRKLLGE ESRISLPLPNFSSLNLRETNLDSLPLVDTHSKRTLLIKTVETRDGQVIN ETSQHDDLE
<u>Native sequence</u>	Amino acids S2 – E466 (end) of human Vimentin. Residue S232 of the fusion protein is equivalent to S2 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccTCCACCAGGTCCGTGTCCTCGTCCTCCTACCGCAGGATGTTTCG
GCGGCCCCGGGCACCGCGAGCCGGCCGAGCTCCAGCCGGAGCTACGTGAC
TACGTCCACCCGCACCTACAGCCTGGGCAGCGCGCTGCGCCCCAGCACC
AGCCGCAGCCTCTACGCCTCGTCCCCGGGCGGCGTGTATGCCACGCGCT
CCTCTGCCGTGCGCCTGCGGAGCAGCGTGCCCGGGGTGCGGCTCCTGCA
GGACTCGGTGGACTTCTCGCTGGCCGACGCCATCAACACCGAGTTCAAG
AACACCCGCACCAACGAGAAGGTGGAGCTGCAGGAGCTGAATGACCGCT
TCGCCAACTACATCGACAAGGTGCGCTTCCTGGAGCAGCAGAATAAGAT
CCTGCTGGCCGAGCTCGAGCAGCTCAAGGGCCAAGGCAAGTCGCGCCTA
GGGGACCTCTACGAGGAGGAGATGCGGGAGCTGCGCCGGCAGGTGGACC
AGCTAACCAACGACAAAGCCCGCGTCGAGGTGGAGCGCGACAACCTGGC
CGAGGACATCATGCGCCTCCGGGAGAAATTGCAGGAGGAGATGCTTCAG
AGAGAGGAAGCCGAAAACACCCTGCAATCTTTCAGACAGGATGTTGACA
ATGCGTCTCTGGCACGTCTTGACCTTGAACGCAAAGTGGAATCTTTGCA
AGAAGAGATTGCCTTTTTTGAAGAACTCCACGAAGAGGAAATCCAGGAG
CTGCAGGCTCAGATTCAGGAACAGCATGTCCAAATCGATGTGGATGTTT
CCAAGCCTGACCTCACGGCTGCCCTGCGTGACGTACGTACGTCAGCAATATGA
AAGTGTGGCTGCCAAGAACCTGCAGGAGGCAGAAGAATGGTACAAATCC
AAGTTTGCTGACCTCTCTGAGGCTGCCAACCAGGAACAATGACGCCCTGC
GCCAGGCAAAGCAGGAGTCCACTGAGTACCGGAGACAGGTGCAGTCCCT
CACCTGTGAAGTGGATGCCCTTAAAGGAACCAATGAGTCCCTGGAACGC
CAGATGCGTGAAATGGAAGAGAACTTTGCCGTTGAAGCTGCTAACTACC
AAGACACTATTGGCCGCCTGCAGGATGAGATTCAGAATATGAAGGAGGA
AATGGCTCGTCACCTTCGTGAATACCAAGACCTGCTCAATGTTAAGATG
GCCCTTGACATTGAGATTGCCACCTACAGGAAGCTGCTGGAAGGCGAGG
AGAGCAGGATTTCTCTGCCTCTTCCAACTTTTCCCTCCCTGAACCTGAG
GGAAACTAATCTGGATTCACTCCCTCTGGTTGATACCCACTCAAAAAGG
ACACTTCTGATTAAGACGGTTGAAACTAGAGATGGACAGGTTATCAACG
AAACTTCTCAGCATCACGATGACCTtgaataaggatcc