

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active VEGFR2 [789 - 1356]

<u>Enzyme description:-</u>	VEGFR2 [789 – 1356]
<u>Clone number:-</u>	DU 26346
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	90,880.67 daltons
Average Mass	90,938.67 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.27

Purity:- >80 %

Activation protocol:-	Constitutively active
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Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSE

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

VEGFR2 [789 - 1356]

<u>Protein</u>	VEGFR2 [781 - 1124]
<u>Clone number</u>	DU 26346
<u>Species</u>	Human
<u>Accession number</u>	NM_002253.2
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSPNSRVD VKR ANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKP LGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELK ILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEF VPYKTKGARFRQGDYVGAI PVDLKRRLDSITSSQSSASSGFVEEKSL SDVEEEEAPEDLYKDFLTLEHLICYSFQVAKGMEFLASRKC IHRDLAA RNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFD RVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRA PDYTTPEMYQTMLDCWHGEP SQRP TFSELVEHLGNLLQANAQQDGKDY IVLP ISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISQY LQNSKRKSRPVSVKTFEDI PLEEPVKVIPDDNQTDSGMVLASEELKT LEDRTKLSPSFGGMVPSKSRESVASEGSNQTSQGYQSGYHSDDTDTTVY SSEEAE LLK LIEIGVQTGSTAQILQPD SGTTLS SPPV
<u>Native sequence</u>	Amino acids V789 – V1356 (end) of human VEGFR2. Residue V238 of the fusion protein is equivalent to V789 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission site (<u>LEVLFQGP</u>) residues 221 – 228
<u>Cloning sites</u>	<i>Sal</i> 1 and <i>Not</i> 1 sites of pFastBac GST 6P3

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Nucleotide sequence of insert

gtcgacGTTAAGCGGGCCAATGGAGGGGAACTGAAGACAGGCTACTTG
TCCATCGTCATGGATCCAGATGAACTCCCATTTGGATGAACATTGTGAA
CGACTGCCTTATGATGCCAGCAAATGGGAATTCCCCAGAGACCGGCTG
AAGCTAGGTAAGCCTCTTGGCCGTGGTGCCTTTGGCCAAGTGATTGAA
GCAGATGCCTTTGGAATTGACAAGACAGCAACTTGCAGGACAGTAGCA
GTCAAAATGTTGAAAGAAGGAGCAACACACAGTGAGCATCGAGCTCTC
ATGTCTGAACTCAAGATCCTCATTCATATTGGTCACCATCTCAATGTG
GTCAACCTTCTAGGTGCCTGTACCAAGCCAGGAGGGCCACTCATGGTG
ATTGTGGAATTCTGCAAATTTGGAAACCTGTCCACTTACCTGAGGAGC
AAGAGAAATGAATTTGTCCCCTACAAGACCAAAGGGGCACGATTCCGT
CAAGGGAAAGACTACGTTGGAGCAATCCCTGTGGATCTGAAACGGCGC
TTGGACAGCATCACCAGTAGCCAGAGCTCAGCCAGCTCTGGATTGTG
GAGGAGAAGTCCCTCAGTGATGTAGAAGAAGAGGAAGCTCCTGAAGAT
CTGTATAAGGACTTCCTGACCTTGGAGCATCTCATCTGTTACAGCTTC
CAAGTGGCTAAGGGCATGGAGTTCTTGGCATCGCGAAAGTGTATCCAC
AGGGACCTGGCGGCACGAAATATCCTCTTATCGGAGAAGAACGTGGTT
AAAATCTGTGACTTTGGCTTGGCCCGGGATATTTATAAAGATCCAGAT
TATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGGATGGCCCCA
GAAACAATTTTTTGACAGAGTGTACACAATCCAGAGTGACGTCTGGTCT
TTTGGTGTTTTGCTGTGGGAAATATTTTCCTTAGGTGCTTCTCCATAT
CCTGGGGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAAGAAGGA
ACTAGAATGAGGGCCCCTGATTATACTACACCAGAAATGTACCAGACC
ATGCTGGACTGCTGGCACGGGGAGCCCAGTCAGAGACCCACGTTTTCA
GAGTTGGTGGAACATTTGGGAAATCTCTTGCAAGCTAATGCTCAGCAG
GATGGCAAAGACTACATTGTTCTTCCGATATCAGAGACTTTGAGCATG
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GGAATCAGTCAGTATCTGCAGAACAGTAAGCGAAAGAGCCGGCCTGTG
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GTAATCCCAGATGACAACCAGACGGACAGTGGTATGGTTCTTGCCTCA
GAAGAGCTGAAAACTTTGAAGACAGAACCAAATTATCTCCATCTTTT
GGTGGAATGGTGCCAGCAAAGCAGGGAGTCTGTGGCATCTGAAGGC
TCAAACCAGACAAGCGGCTACCAGTCCGGATATCACTCCGATGACACA
GACACCACCGTGTACTCCAGTGAGGAAGCAGAACTTTTAAAGCTGATA
GAGATTGGAGTGCAAACCGGTAGCACAGCCCAGATTCTCCAGCCTGAC
TCGGGGACCACACTGAGCTCTCCTCCTGTTtagcggccgc