

Standard Operating Procedure

**Preparation of Vascular Endothelial Growth Factor Receptor
[784 - 1338]**

Enzyme description:- VEGFR [784 - 1338]

Clone number:- DU 8999

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 89, 588.98 daltons

Average Mass 89, 646.69 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.03

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

Assay buffer:-

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

Substrate:-

KKKSPGEYVNIEFG Final concentration: 300 µM

Specific activity range:- To be determined

Clone Data Sheet - Vascular Endothelial Growth Factor Receptor [784 - 1338]

<u>Protein</u>	VEGFR [784 - 1338]
<u>Clone number</u>	DU 8999
<u>Species</u>	Human
<u>Accession number</u>	NM_002019.3
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFE DRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDL <u>EVLFQGPLGSKRSSEIKTDYLSIIMDPDEVPLDEQCERLPYDA</u> <u>SKWEFARERLKLGKSLGRGAFGKVVQASAFGIKKSPTCRTV</u> AVKMLKEGATASEYKALMTELKILTHIGHHLNVNLLGACT KQGGPLMVIVEYCKYGNLSNYLKSKRDLFFLNKDAALHMEP KKEKMEPGLEQGKKPRLDSVTSSSFASSGFQEDKSLSDVEE EEDSDGFYKEPITMEDLISYSFQVARGMEFLSSRKCIHRDLAA RNILLSENNVVKICDFGLARDIYKNPDYVRKGDTRLPLKWMA PESIFDKIYSTKSDVWSYGVLLWEIFSLGGSPYPGVQMDDEFC SRLREGMRMRAP EYSTPEIYQIMLDCWHRDPKERPRFAELV EKLGDLLQANVQQDGKDYIPINAILTGNSGFTYSTPAFSEDF KESISAPKFNSGSSDDVRYVNAFKFMSLERIKTFEELLPNATS MFDDYQGDSSTLLASPMLKRFTWTDSKPKASLKIDLRVTSKS KESGLSDVSRPSFCHSSCGHVSEGKRRFTYDHAELERK IACCS PPPDYNSVVLYSTPPI
<u>Native sequence</u>	Amino acids K784 – I1338 (end) of human VEGFR. Residue K232 of the fusion protein is equivalent to K784 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVLFQGPL) residues 221 - 229
<u>Cloning sites</u>	BamH1 and Not1 sites of pFastBAC GEX6P-1

Nucleotide
sequence of insert

ggatccAAAAGGTCTTCTTCTGAAATAAAGACTGACTACCTATCA
ATTATAATGGACCCAGATGAAGTTCCTTTGGATGAGCAGTGTG
AGCGGCTCCCTTATGATGCCAGCAAGTGGGAGTTTGCCCGGG
AGAGACTTAAACTGGGCAAATCACTTGAAGAGGGGGCTTTTG
GAAAAGTGGTTCAAGCATCAGCATTTGGCATTAAAGAAATCAC
CTACGTGCCGGACTGTGGCTGTGAAAATGCTGAAAGAGGGGG
CCACGGCCAGCGAGTACAAAGCTCTGATGACTGAGCTAAAAA
TCTTGACCCACATTGGCCACCATCTGAACGTGGTTAACCTGCT
GGGAGCCTGCACCAAGCAAGGAGGGCCTCTGATGGTGATTGT
TGAATACTGCAAATATGGAAATCTCTCCAACCTCAAGAG
CAAACGTGACTTATTTTTTCTCAACAAGGATGCAGCACTACAC
ATGGAGCCTAAGAAAGAAAAAATGGAGCCAGGCCTGGAACA
AGGCAAGAAACCAAGACTAGATAGCGTCACCAGCAGCGAAA
GCTTTGCGAGCTCCGGCTTTCAGGAAGATAAAAGTCTGAGTG
ATGTTGAGGAAGAGGAGGATTCTGACGGTTTCTACAAGGAGC
CCATCACTATGGAAGATCTGATTTCTTACAGTTTCAAGTGGC
CAGAGGCATGGAGTTCCTGTCTTCCAGAAAGTGCATTCATCGG
GACCTGGCAGCGAGAAACATTCTTTTATCTGAGAACAACGTG
GTGAAGATTTGTGATTTTGGCCTTGCCCGGGATATTTATAAGA
ACCCCGATTATGTGAGAAAAGGAGATACTCGACTTCCTCTGA
AATGGATGGCTCCTGAATCTATCTTTGACAAAATCTACAGCAC
CAAGAGCGACGTGTGGTCTTACGGAGTATTGCTGTGGGAAAT
CTTCTCCTTAGGTGGGTCTCCATACCCAGGAGTACAAATGGAT
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AACAGGATGGTAAAGACTACATCCCAATCAATGCCATACTGA
CAGGAAATAGTGGGTTTACATACTCAACTCCTGCCTTCTCTGA
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ATGCCACCTCCATGTTTGATGACTACCAGGGCGACAGCAGCA
CTCTGTTGGCCTCTCCCATGCTGAAGCGCTTACCTGGACTGA
CAGCAAACCCAAGGCCTCGCTCAAGATTGACTTGAGAGTAAC
CAGTAAAAGTAAGGAGTCGGGGCTGTCTGATGTCAGCAGGCC
CAGTTTCTGCCACTCCAGCTGTGGGCACGTCAGCGAAGGCAA
GCGCAGGTTACCTACGACCACGCTGAGCTGGAAAGGAAAAT
CGCGTGCTGCTCCCCGCCCCCAGACTACAACCTCGGTGGTCTCTG
TACTCCACCCAC CCATCtaggcggccgcg