Certificate of Analysis

REACTION BIOLOGY

ProQinase™ VEGFR1

fms related receptor tyrosine kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: FLT1

Synonyms: FLT, VEGF-R1

Product No.: 0097-0000-3

Lot: 011

Description: Human VEGFR1, C-terminal fragment, amino acids K_{784}-I_{1338} (as in NCBI/Protein entry NP_002010.1), N-terminal GST-HIS_6 fusion protein with a Factor Xa cleavage site, expressed in Sf9 insect cells

Product identity: VEGFR1 LOT011, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 89357 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

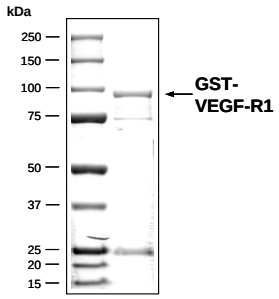
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.16 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

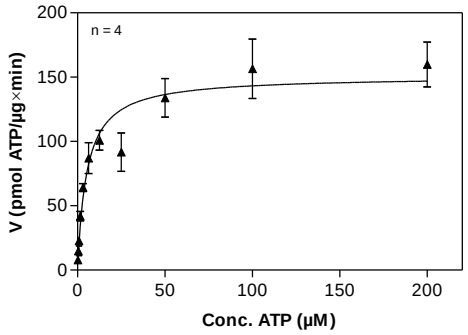
Biochemical Parameters:
Specific kinase activity (P_i transfer): 151 pmol/µg*min
ATP-K_M: 5.2 µM

VEGFR1 LOT 011:
Coomassie stain



2.0 µg GST-VEGF-R1

VEGFR1 LOT 011:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: $Poly(Glu:Tyr)_{4:1}$ 40 µg/ml
  Kinase: 2 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

REACTION BIOLOGY

Sequence information

GST-VEGFR1 Recombinant Fusion Protein Amino Acid Sequence

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LIEGRGILKR SSSEIKTDYL 240
241 SIIMDPDEVP LDEQCERLPY DASKWEFARE RLKLGKSLGR GAFGKVVQAS AFGIKKSPTC 300
301 RTVAVKMLKE GATASEYKAL MTELKILTHI GHHLNVVNLL GACTKQGGPL MVIVEYCKYG 360
361 NLSNYLKSKR DLFFLNKDAA LHMEPKKEKM EPGLEQGKKP RLDSVTSSES FASSGFQEDK 420
421 SLSDVEEEED SDGFYKEPIT MEDLISYSFQ VARGMEFLSS RKCIHRDLAA RNILLSENNV 480
481 VKICDFGLAR DIYKNPDYVR KGDTRLPLKW MAPESIFDKI YSTKSDVWSY GVLLWEIFSL 540
541 GGSPYPGVQM DEDFCSRLRE GMRMRAPEYS TPEIYQIMLD CWHRDPKERP RFAELVEKLG 600
601 DLLQANVQQD GKDYIPINAI LTGNSGFTYS TPAFSEDFFK ESISAPKFNS GSSDDVRYVN 660
661 AFKFMSLERI KTFEELLPNA TSMFDDYQGD SSTLLASPML KRFTWTDSKP KASLKIDLRV 720
721 TSKSKESGLS DVSRPSFCHS SCGHVSEGKR RFTYDHAELE RKIACCSPPP DYNSVVLYST 780
781 PPI                                                              840
```

1-218: GST Pink: Factor Xa cleavage site blue: VEGFR1 fragment

VEGFR1 wt[1] Amino Acid Sequence

```
  1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK  60
 61 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET 120
121 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD 180
181 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV 240
241 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK 300
301 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK 360
361 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA 420
421 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC 480
481 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK 540
541 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM 600
601 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRDQEA 660
661 PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK IQQEPGIILG PGSSTLFIER 720
721 VTEEDEGVYH CKATNQKGSV ESSAYLTVQG TSDKSNLELI TLTCTCVAAT LFWLLLTLLI 780
781 RKMKRSSSEI KTDYLSIIMD PDEVPLDEQC ERLPYDASKW EFARERLKLG KSLGRGAFGK 840
841 VVQASAFGIK KSPTCRTVAV KMLKEGATAS EYKALMTELK ILTHIGHHLN VVNLLGACTK 900
901 QGGPLMVIVE YCKYGNLSNY LKSKRDLFFL NKDAALHMEP KKEKMEPGLE QGKKPRLDSV 960
961 TSSESFASSG FQEDKSLSDV EEEEDSDGFY KEPITMEDLI SYSFQVARGM EFLSSRKCIH 1020
1021 RDLAARNILL SENNVVKICD FGLARDIYKN PDYVRKGDTR LPLKWMAPES IFDKIYSTKS 1080
1081 DVWSYGVLLW EIFSLGGSPY PGVQMDEDFC SRLREGMRMR APEYSTPEIY QIMLDCWHRD 1140
1141 PKERPRFAEL VEKLGDLLQA NVQQDGKDYI PINAILTGNS GFTYSTPAFS EDFFKESISA 1200
1201 PKFNSGSSDD VRYVNAFKFM SLERIKTFEE LLPNATSMFD DYQGDSSTLL ASPMLKRFTW 1260
1261 TDSKPKASLK IDLRVTSKSK ESGLSDVSRP SFCHSSCGHV SEGKRRFTYD HAELERKIAC 1320
1321 CSPPPDYNSV VLYSTPPI                                              1380
```

blue: VEGFR1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002010.1

Recombinant Proteins