Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

FGF-R1 wt

fibroblast growth factor receptor 1

Recombinant Human Active Protein Kinase

HGNC Symbol: FGFR1

Synonyms: BFGFR, N-SAM, CEK, FLT2, FLG, CD133, H2, H3, H4, H5

Product No.: 0101-0000-1

Lot: 011

Description: Human FGF-R1, C-terminal fragment, amino acids G_{400}-R_{820} (as in NCBI/Protein entry NP_056934.2), N-terminally fused to GST-HIS_6-Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: FGF-R1 wt, Lot 011, was confirmed as human FGF-R1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 78,097 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

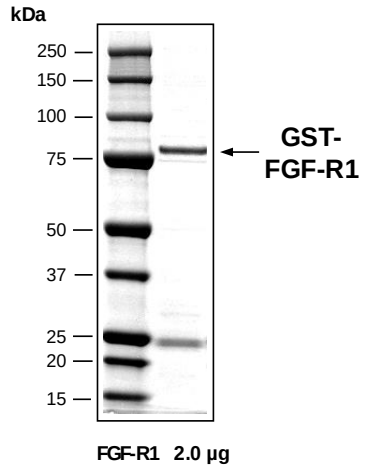
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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!

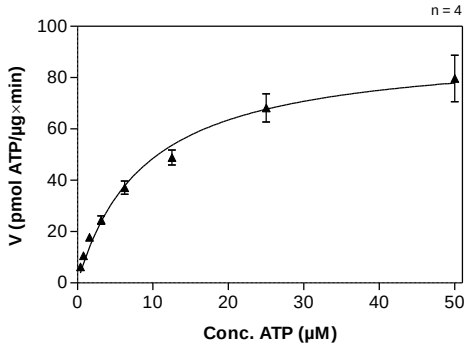
Protein concentration: 0.107 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 92 pmol/µg × min
ATP-K_M: 8.9 µM

FGF-R1 wt Lot 011:
Coomassie stain



FGF-R1 wt Lot 011:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- 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 10 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
FGF-R1 wt Lot 011 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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FGF-R1 wt

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GST-FGF-R1 wt 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 PMGHHHHHHG RRRASVAAGI	240
241	LVPRGSPGLD GIYARGIQAS MGARGRLQGT KKSDFHSQMA VHKLAKSIPL RRQVTVSADS	300
301	SASMNSGVLL VRPSRLSSSG TPMLAGVSEY ELPEDLRWEL PRDRLVLGKP LGEGCFGQVV	360
361	LAEAIGLDKD KPNRVTKVAV KMLKSDATEK DLSDLISEME MMKMIGKHKN IINLLGACTQ	420
421	DGPLYVIVEY ASKGNLREYL QARRPPGLEY CYNPSHNPEE QLSSKDLVSC AYQVARGMEY	480
481	LASKKCIHRD LAARNVLVTE DNVMKIADFG LARDIHHIDY YKKTTNGRLP VKWMAPEALF	540
541	DRIYTHQSDV WSFGVLLWEI FTLGGSPYPG VPVEELFKLL KEGHRMDKPS NCTNELYMMM	600
600	RDCWHAVPSQ RPTFKQLVED LDRIVALTSN QEYLDLSMPL DQYSPSFPDT RSSTCSSGED	660
661	SVFSHEPLPE EPCLPRHPAQ LANGGLKRR	720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: FGF-R1 fragment **boxed**: variation from RefSeq

FGF-R1 wt[1] Amino Acid Sequence

1	MWSWKCLLFW AVLVTATLCT ARPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD	60
61	VQSINWLRDG VQLAESNRTR ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD	120
121	ALPSSEDDDD DDDSSSEEKE TDNTKPNPVA PYWTSPEKME KKLHAVPAAK TVKFKCPSSG	180
181	TPNPTLRWLK NGKEFKPDHR IGGYKVRYAT WSIIMDSVVP SDKGNYTCIV ENEYGSINHT	240
241	YQLDVVERSP HRPILQAGLP ANKTVALGSN VEFMCKVYSD PQPHIQWLKH IEVNGSKIGP	300
301	DNLPYVQILK TAGVNTTDKE MEVLHLRNVS FEDAGEYTCL AGNSIGLSHH SAWLTVLEAL	360
361	EERPAVMTSP LYLEIIIYCT GAFLISCMVG SVIVYKMKSG TKKSDFHSQM AVHKLAKSIP	420
421	LRRQVTVSAD SSASMNSGVL LVRPSRLSSS GTPMLAGVSE YELPEDPRWE LPRDRLVLGK	480
481	PLGEGCFGQV VLAEAIGLDK DKPNRVTKVA VKMLKSDATE KDLSDLISEM EMMKMIGKHK	540
541	NIINLLGACT QDGPLYVIVE YASKGNLREY LQARRPPGLE YCYNPSHNPE EQLSSKDLVS	600
600	CAYQVARGME YLASKKCIHR DLAARNVLVT EDNVMKIADF GLARDIHHID YYKKTTNGRL	660
661	PVKWMAPEAL FDRIYTHQSD VWSFGVLLWE IFTLGGSPYP GVPVEELFKL LKEGHRMDKP	720
721	SNCTNELYMM MRDCWHAVPS QRPTFKQLVE DLDRIVALTS NQEYLDLSMP LDQYSPSFPD	780
781	TRSSTCSSGE DSVFSHEPLP EEPCLPRHPA QLANGGLKRR	840

blue: FGF-R1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_056934.2
P467L: Sequence conflict: see UniProt P11362 and GenBank M37722.1

Recombinant Proteins