Certificate of Analysis

REACTION BIOLOGY

ProQinase™ FGF-R3 K650M

fibroblast growth factor receptor 3

Recombinant Human Active Protein Kinase

HGNC Symbol: FGFR3

Synonyms: ACH, CD333, CEK2, FGFR-3, HSFGFR3EX, JTK4

Product No.: 1069-0000-1

Lot: 001

Description: Human FGF-R3, C-terminal fragment, amino acids R_{397}-T_{806} (as in NCBI/Protein entry NP_000133.1) with a K650M mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: FGF-R3 K650M, Lot 001, was confirmed as FGF-R3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 73,937 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

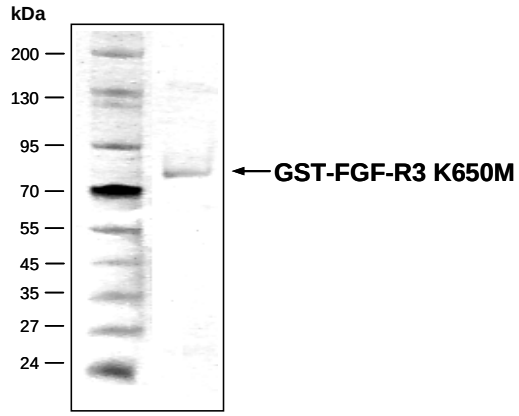
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

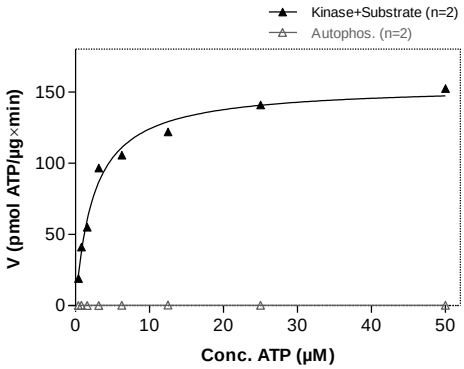
Biochemical Parameters:
Specific kinase activity (P_i transfer): 154 pmol/µg × min
ATP-K_M: 2.5 µM

FGF-R3 K650M Lot 001: Coomassie stain



2.0 µg GST-FGF-R3 K650M

FGF-R3 K650M Lot 001: 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: TRK-C-derived peptide, 40 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
FGF-R3 K650M Lot 001 was also successfully tested by Reaction Biology 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

Recombinant Proteins

REACTION BIOLOGY

ProQinase™ FGF-R3 K650M

Product No.: 01069-0000-1

GST-FGF-R3 K650M Recombinant Fusion Protein Amino Acid Sequence

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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 RDSLEVLFQG 240
241  PLAMVRLRSP PKKGLGSPTV HKISRFPLKR QVSLESNASM SSNTPLVRIA RLSSGEGPTL 300
301  ANVSELELPA DPKWELSRAR LTLGKPLGEG CFGQVVMAEA IGIDKDRAAK PVTVAVKMLK 360
361  DDATDKDLSD LVSEMEMMKM IGKHKNIINL LGACTQGGPL YVLVEYAAKG NLREFLRARR 420
421  PPGLDYSFDT CKPPEEQLTF KDLVSCAYQV ARGMEYLASQ KCIHRDLAAR NVLVTEDNVM 480
481  KIADFGLARD VHNLDYYKMT TNGRLPVKWM APEALFDRVY THQSDVWSFG VLLWEIFTLG 540
541  GSPYPGIPVE ELFKLLKEGH RMDKPANCTH DLYMIMRECW HAAPSQRPTF KQLVEDLDRV 600
600  LTVTSTDEYL DLSAPFEQYS PGGQDTPSSS SSGDDSVFAH DLLPPAPPSS GGSRT     660
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: FGF-R3 fragment **boxed**: K650M

FGF-R3 wt[1] Amino Acid Sequence

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  1  MGAPACALAL CVAVAIVAGA SSESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS  60
 61  CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH 120
121  FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG 180
181  NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT 240
241  YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP 300
301  DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE 360
361  EELVEADEAG SVYAGILSYG VGFFLFILVV AAVTLCRLRS PPKKGLGSPT VHKISRFPLK 420
421  RQVSLESNAS MSSNTPLVRI ARLSSGEGPT LANVSELELP ADPKWELSRA RLTLGKPLGE 480
481  GCFGQVVMAE AIGIDKDRAA KPVTVAVKML KDDATDKDLS DLVSEMEMMK MIGKHKNIIN 540
541  LLGACTQGGP LYVLVEYAAK GNLREFLRAR RPPGLDYSFD TCKPPEEQLT FKDLVSCAYQ 600
600  VARGMEYLAS QKCIHRDLAA RNVLVTEDNV MKIADFGLAR DVHNLDYYKK TTNGRLPVKW 660
661  MAPEALFDRV YTHQSDVWSF GVLLWEIFTL GGSPYPGIPV EELFKLLKEG HRMDKPANCT 720
721  HDLYMIMREC WHAAPSQRPT FKQLVEDLDR VLTVTSTDEY LDLSAPFEQY SPGGQDTPSS 780
781  SSSGDDSVFA HDLLPPAPPS SGGSRT                                      840
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blue: FGF-R3 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_000133.1

Recombinant Proteins