Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

FER

FER tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: FER

Synonyms: TYK3, c-FER, p94-FER, PPP1R74

Product No.: 0533-0000-1

Lot: 002

Description: Human FER, C-terminal fragment, amino acids K_{541}-T_{822} (as in NCBI/Protein entry NP_005237.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in insect cells

Product identity: FER, Lot 002, was confirmed as FER by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 61,733 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

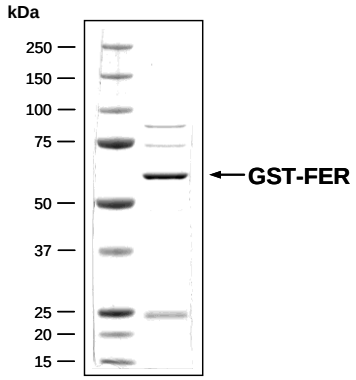
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

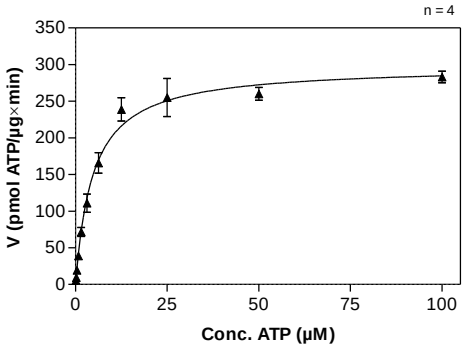
Biochemical Parameters:
Specific kinase activity (P_i transfer): 298 pmol/µg × min
ATP-K_M: 4.7 µM

FER Lot 002:
Coomassie stain



2.0 µg GST-FER

FER Lot 002:
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: $Poly(Glu,Tyr)_{4:1}$ 10 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
FER Lot 002 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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GST-FER 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 GICSRNSKSG VVLLNPIPKD KKWILSHEDV ILGELLGKGN FGEVYKGTLK	300
301	DKTSVAVKTC KEDLPQELKI KFLQEAKILK QYDHPNIVKL IGVCTQRQPV YIIMELVSGG	360
361	DFLTFLRRKK DELKLKQLVK FSLDAAAGML YLESKNCIHR DLAARNCLVG ENNVLKISDF	420
421	GMSRQEDGGV YSSSGLKQIP IKWTAPEALN YGRYSSESDV WSFGILLWET FSLGVCPYPG	480
481	MTNQQAREQV ERGYRMSAPQ HCPEDISKIM MKCWDYKPEN RPKFSELQKE LTIIKRKLT	540

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: FER fragment

FER wt[1] Amino Acid Sequence

1	MGFGSDLKNS HEAVLKLQDW ELRLLETVKK FMALRIKSDK EYASTLQNLC NQVDKESTVQ	60
61	MNYVSNVSKS WLLMIQQTEQ LSRIMKTHAE DLNSGPLHRL TMMIKDKQQV KKSYIGVHQQ	120
121	IEAEMIKVTK TELEKLKCSY RQLIKEMNSA KEKYKEALAK GKETEKAKER YDKATMKLHM	180
181	LHNQYVLALK GAQLHQNQYY DITLPLLLDS LQKMQEEMIK ALKGIFDEYS QITSLVTEEI	240
241	VNVHKEIQMS VEQIDPSTEY NNFIDVHRTT AAKEQEIEFD TSLLEENENL QANEIMWNNL	300
301	TAESLQVMLK TLAEELMQTQ QMLLNKEEAV LELEKRIEES SETCEKKSDI VLLLSQKQAL	360
361	EELKQSVQQL RCTEAKFSAQ KELLEQKVQE NDGKEPPPVV NYEEDARSVT SMERKERLSK	420
421	FESIRHSIAG IIRSPKSAVG SSALSDMISI SEKPLAEQDW YHGAIPRIEA QELLKKQGDF	480
481	LVRESHGKPG EYVLSVYSDG QRRHFIIQYV DNMYRFEGTG FSNIPQLIDH HYTTKQVITK	540
541	KSGVVLLNPI PKDKKWILSH EDVILGELLG KGNFGEVYKG TLKDKTSVAV KTCKEDLPQE	600
600	LKIKFLQEAK ILKQYDHPNI VKLIGVCTQR QPVYIIMELV SGGDFLTFLR RKKDELKLKQ	660
661	LVKFSLDAAA GMLYLESKNC IHRDLAARNC LVGENNVLKI SDFGMSRQED GGVYSSSGLK	720
721	QIPIKWTAPE ALNYGRYSSE SDVWSFGILL WETFSLGVCP YPGMTNQQAR EQVERGYRMS	780
781	APQHCPEDIS KIMMKCWDYK PENRPKFSEL QKELTIIKRK LT	840

blue: FER sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005237.1

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