

Certificate of Analysis

FRK

fyn-related kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: FRK

Synonyms: GTK, PTK5, RAK

Product No.: 0446-0000-1

Lot: 001

Description: Human FRK, full length, amino acids M₁-R₅₀₅ (as in NCBI/Protein entry NP_002022.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

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

Theoretical MW_{Fusion Protein}: 87,649 Da

Expression: Baculovirus infected Sf9 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!

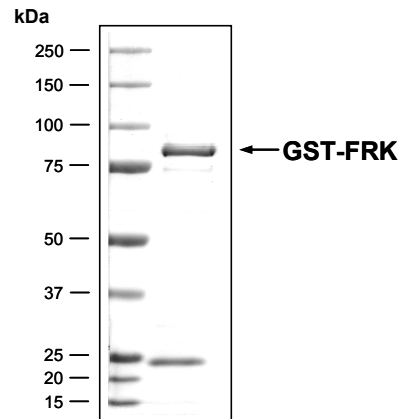
Protein concentration: 0.098 µg/µl

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

Biochemical Parameters:

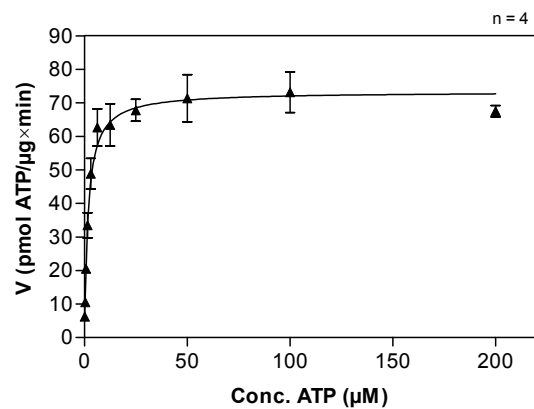
Specific kinase activity (P_i transfer): 73 pmol/µg × min
ATP-K_M: 1.8 µM

FRK Lot 001:
Coomassie stain



2.0 µg GST-FRK

FRK 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 MgCl₂
 - 3 mM MnCl₂
 - 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: 4.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: FRK Lot 001

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

FRK

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FRK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPPYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GICSRMSNIC	QRLWEYLEPY	LPCLSTEADK	STVIENPGAL	CSPQSQRHGH	300
301	YFVALFDYQA	RTAEDLSFRA	GDKLQVLDL	HEGWWFARHL	EKRRDGSSQQ	LQGYIPSNYV	360
361	AEDRSLOAEP	WFFGAIGRSD	AEKQLLYSEN	KTGSFLIRES	ESQKGEFSL	VLDGAVVKHY	420
421	RIKRLDEGGF	FLTRRRIFST	LNEFVSHYTK	TSDGLCVKLG	KPCLKIQVPA	PFDLSYKTVD	480
481	QWEIDRNSIQ	LLKRLGSGQF	GEVWEGLWNN	TTPVAVKTLK	PGSMDPNDFL	REAQIMKNLR	540
541	HPKLIQLYAV	CTLEDPIYII	TELMRHGSLQ	EYLQNDTGSK	IHLTQOVDMA	AQVASGMAYL	600
601	ESRNYIHRDL	AARNVLGEH	NIYKVADFL	ARVFKVDNED	IYESRHEIKL	PVKWTAPEAI	660
661	RSNKFSIKSD	VWSFGILLYE	IITYGKMPYS	GMTGAQVIQM	LAQNYRLPQP	SNCPPQFYNI	720
721	MLECWNAEPK	ERPTFETLRW	KLEDYFETDS	SYSDANNFIR			780

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

FRK wt ¹ Amino Acid Sequence							
1	MSNICQRLWE	YLEPYLPCLS	TEADKSTVIE	NPGALCSPQS	QRHGHYFVAL	FDYQARTAED	60
61	LSFRAGDKLQ	VLDTLHEGW	FARHLEKRRD	GSSQQLQGYI	PSNYVAEDRS	LQAEPWFFGA	120
121	IGRSDAEKQL	LYSENKTGSF	LIRESESQKG	EFSLSVLDGA	VVKHYRIKRL	DEGGFFLTRR	180
181	RIFSTLNEFV	SHYTKTSDGL	CVKLGKPKLK	IQVPAPFDLS	YKTVDQWEID	RNSIQLLKRL	240
241	GSGQFGEVWE	GLWNNTTPVA	VKTLKPGSMD	PNDFLREAQI	MKNLRHPKLI	QLYAVCTLED	300
301	PIYIITELMR	HGSLQEYLQ	DTGSKIHLTQ	QVDMAAQVAS	GMAYLESRNY	IHRDLAARNV	360
361	LVGEHNIYKV	ADFLARVFK	VDNEDIYESR	HEIKLPVKWT	APEAIRSNKF	SIKSDVWSFG	420
421	ILLYEIIITYG	KMPYSGMTGA	QVIQMLAQNY	RLPQPSNCPQ	QFYNIMLECW	NAEPKERPTF	480
481	ETLRWKLEDY	FETDSSYSDA	NNFIR				540

blue: FRK sequence expressed in fusion protein

¹NCBI/Protein accession number NP_002022.1