Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

ITK

IL2 inducible T cell kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: ITK

Synonyms: EMT; LYK; PSCTK2

Product No.: 0359-0000-1

Lot: 001

Description: Human ITK, full length, amino acids M_1-L_{630} (as in NCBI/Protein entry NP_005537.3), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

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

Theoretical $MW_{Fusion\ Protein}$: 101,727 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

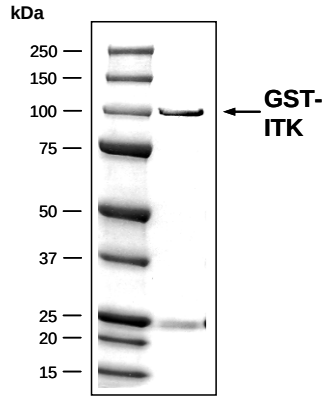
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

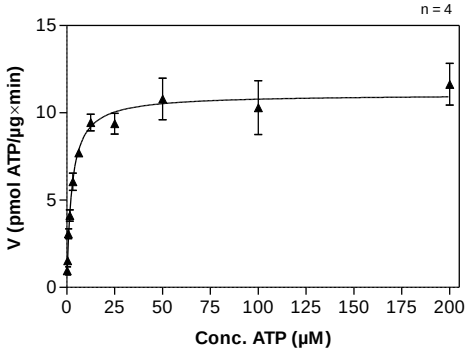
Biochemical Parameters:
Specific kinase activity (P_i transfer): 11 pmol/µg × min
ATP-K_M: 2.5 µM

ITK Lot 001:
Coomassie stain



2.0 µg GST-ITK

ITK Lot 001:
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)$_{4:1}$, 20 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

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GST-ITK 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 MNNFILLEEQ LIKKSQQKRR TSPSNFKVRF FVLTKASLAY	300
301	FEDRHGKKRT LKGSIELSRI KCVEIVKSDI SIPCHYKYPF QVVHDNYLLY VFAPDRESRQ	360
361	RWVLALKEET RNNNSLVPKY HPNFWMDGKW RCCSQLEKLA TGCAQYDPTK NASKKPLPPT	420
421	PEDNRRPLWE PEETVVIALY DYQTNDPQEL ALRRNEEYCL LDSSEIHWWR VQDRNGHEGY	480
481	VPSSYLVEKS PNNLETYEWY NKSISRDKAE KLLLDTGKEG AFMVRDSRTA GTYTVSVFTK	540
541	AVVSENNPCI KHYHIKETND NPKRYYVAEK YVFDSIPLLI NYHQHNGGGL VTRLRYPVCF	600
601	GRQKAPVTAG LRYGKWVIDP SELTFVQEIG SGQFGLVHLG YWLNKDKVAI KTIREGAMSE	660
661	EDFIEEAEVM MKLSHPKLVQ LYGVCLEQAP ICLVFEFMEH GCLSDYLRTQ RGLFAAETLL	720
721	GMCLDVCEGM AYLEEACVIH RDLAARNCLV GENQVIKVSD FGMTRFVLDD QYTSSTGTKF	780
781	PVKWASPEVF SFSRYSSKSD VWSFGVLMWE VFSEGKIPYE NRSNSEVVED ISTGFRLYKP	840
841	RLASTHVYQI MNHCWKERPE DRPAFSRLLR QLAEIAESGL	900

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

ITK wt[1] Amino Acid Sequence

1	MNNFILLEEQ LIKKSQQKRR TSPSNFKVRF FVLTKASLAY FEDRHGKKRT LKGSIELSRI	60
61	KCVEIVKSDI SIPCHYKYPF QVVHDNYLLY VFAPDRESRQ RWVLALKEET RNNNSLVPKY	120
121	HPNFWMDGKW RCCSQLEKLA TGCAQYDPTK NASKKPLPPT PEDNRRPLWE PEETVVIALY	180
181	DYQTNDPQEL ALRRNEEYCL LDSSEIHWWR VQDRNGHEGY VPSSYLVEKS PNNLETYEWY	240
241	NKSISRDKAE KLLLDTGKEG AFMVRDSRTA GTYTVSVFTK AVVSENNPCI KHYHIKETND	300
301	NPKRYYVAEK YVFDSIPLLI NYHQHNGGGL VTRLRYPVCF GRQKAPVTAG LRYGKWVIDP	360
361	SELTFVQEIG SGQFGLVHLG YWLNKDKVAI KTIREGAMSE EDFIEEAEVM MKLSHPKLVQ	420
421	LYGVCLEQAP ICLVFEFMEH GCLSDYLRTQ RGLFAAETLL GMCLDVCEGM AYLEEACVIH	480
481	RDLAARNCLV GENQVIKVSD FGMTRFVLDD QYTSSTGTKF PVKWASPEVF SFSRYSSKSD	540
541	VWSFGVLMWE VFSEGKIPYE NRSNSEVVED ISTGFRLYKP RLASTHVYQI MNHCWKERPE	600
601	DRPAFSRLLR QLAEIAESGL	660

blue: ITK sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005537.3