Certificate of Analysis

REACTION BIOLOGY

ProQinase™ ALK L1196M (GST-HIS-tag)

ALK receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: ALK

Synonyms: CD246

Product No.: 1180-0000-1

Lot: 001

Description: Human ALK L1196M, internal fragment, amino acids L_{1066}-S_{1437} (as in NCBI/Protein entry NP_004295.2), L1196M mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ALK L1196M Lot 001, was confirmed as ALK with a L1196M mutation by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 70,477 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

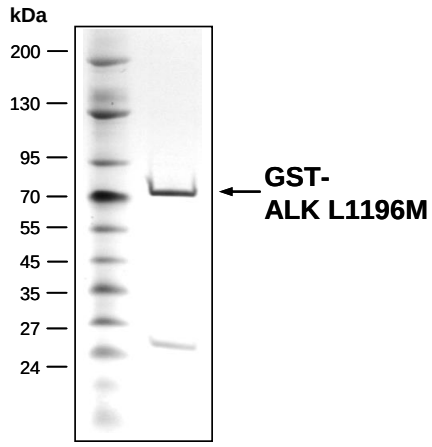
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

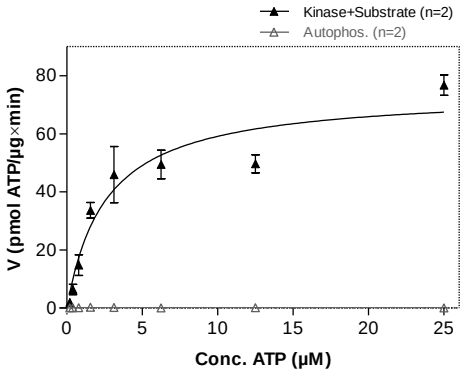
Biochemical Parameters:
Specific kinase activity (P_i transfer): 74 pmol/µg × min
ATP-K_M: 2.6 µM

ALK L1196M Lot 001:
Coomassie stain



2.0 µg GST-ALK L1196M

ALK L1196M 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, 20 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
ALK L1196M 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

ProQinase™ ALK L1196M (GST-HIS-tag)

Product No.: 1180-0000-1

Recombinant Proteins

GST-ALK L1196M 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 PLAMVLQAMQ MELQSPEYKL SKLRTSTIMT DYNPNYCFAG KTSSISDLKE VPRKNITLIR 300
301 GLGHGAFGEV YEGQVSGMPN DPSPLQVAVK TLPEVCSEQD ELDFLMEALI ISKFNHQNIV 360
361 RCIGVSLQSL PRFILMELMA GGDLKSFLRE TRPRPSQPSS LAMLDLLHVA RDIACGCQYL 420
421 EENHFIHRDI AARNCLLTCP GPGRVAKIGD FGMARDIYRA SYYRKGGCAM LPVKWMPPEA 480
481 FMEGIFTSKT DTWSFGVLLW EIFSLGYMPY PSKSNQEVLE FVTSGGRMDP PKNCPGPVYR 540
541 IMTQCWQHQP EDRPNFAIIL ERIEYCTQDP DVINTALPIE YGPLVEEEEK VPVRPKDPEG 600
600 VPPLLVSQQA KREEERS                                              660
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: ALK fragment **boxed**: L1196M

ALK wt[1] Amino Acid Sequence

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  1 MGAIGLLWLL PLLLSTAAVG SGMGTGQRAG SPAAGPPLQP REPLSYSRLQ RKSLAVDFVV  60
 61 PSLFRVYARD LLLPPSSSEL KAGRPEARGS LALDCAPLLR LLGPAPGVSW TAGSPAPAEA 120
121 RTLSRVLKGG SVRKLRRAKQ LVLELGEEAI LEGCVGPPGE AAVGLLQFNL SELFSWWIRQ 180
181 GEGRLRIRLM PEKKASEVGR EGRLSAAIRA SQPRLLFQIF GTGHSSLESP TNMPSPSPDY 240
241 FTWNLTWIMK DSFPFLSHRS RYGLECSFDF PCELEYSPPL HDLRNQSWSW RRIPSEEASQ 300
301 MDLLDGPGAE RSKEMPRGSF LLLNTSADSK HTILSPWMRS SSEHCTLAVS VHRHLQPSGR 360
361 YIAQLLPHNE AAREILLMPT PGKHGWTVLQ GRIGRPDNPF RVALEYISSG NRSLSAVDFF 420
421 ALKNCSEGTS PGSKMALQSS FTCWNGTVLQ LGQACDFHQD CAQGEDESQM CRKLPVGFYC 480
481 NFEDGFCGWT QGTLSPHTPQ WQVRTLKDAR FQDHQDHALL LSTTDVPASE SATVTSATFP 540
541 APIKSSPCEL RMSWLIRGVL RGNVSLVLVE NKTGKEQGRM VWHVAAYEGL SLWQWMVLPL 600
600 LDVSDRFWLQ MVAWWGQGSR AIVAFDNISI SLDCYLTISG EDKILQNTAP KSRNLFERNP 660
661 NKELKPGENS PRQTPIFDPT VHWLFTTCGA SGPHGPTQAQ CNNAYQNSNL SVEVGSEGPL 720
721 KGIQIWKVPA TDTYSISGYG AAGGKGGKNT MMRSHGVSVL GIFNLEKDDM LYILVGQQGE 780
781 DACPSTNQLI QKVCIGENNV IEEEIRVNRS VHEWAGGGGG GGGATYVFKM KDGVPVPLII 840
841 AAGGGGRAYG AKTDTFHPER LENNSSVLGL NGNSGAAGGG GGWNDNTSLL WAGKSLQEGA 900
901 TGGHSCPQAM KKWGWETRGG FGGGGGGCSS GGGGGGYIGG NAASNNDPEM DGEDGVSFIS 960
961 PLGILYTPAL KVMEGHGEVN IKHYLNCSHC EVDECHMDPE SHKVICFCDH GTVLAEDGVS 1020
1021 CIVSPTPEPH LPLSLILSVV TSALVAALVL AFSGIMIVYR RKHQELQAMQ MELQSPEYKL 1080
1081 SKLRTSTIMT DYNPNYCFAG KTSSISDLKE VPRKNITLIR GLGHGAFGEV YEGQVSGMPN 1140
1141 DPSPLQVAVK TLPEVCSEQD ELDFLMEALI ISKFNHQNIV RCIGVSLQSL PRFILLELMA 1200
1201 GGDLKSFLRE TRPRPSQPSS LAMLDLLHVA RDIACGCQYL EENHFIHRDI AARNCLLTCP 1260
1261 GPGRVAKIGD FGMARDIYRA SYYRKGGCAM LPVKWMPPEA FMEGIFTSKT DTWSFGVLLW 1320
1321 EIFSLGYMPY PSKSNQEVLE FVTSGGRMDP PKNCPGPVYR IMTQCWQHQP EDRPNFAIIL 1380
1381 ERIEYCTQDP DVINTALPIE YGPLVEEEEK VPVRPKDPEG VPPLLVSQQA KREEERSPAA 1440
1441 PPPLPTTSSG KAAKKPTAAE ISVRVPRGPA VEGGHVNMAF SQSNPPSELH KVHGSRNKPT 1500
1501 SLWNPTYGSW FTEKPTKKNN PIAKKEPHDR GNLGLEGSCT VPPNVATGRL PGASLLLEPS 1560
1561 SLTANMKEVP LFRLRHFPCG NVNYGYQQQG LPLEAATAPG AGHYEDTILK SKNSMNQPGP 1620
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blue: ALK sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_004295.2