Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ ALK G1202R

ALK receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: ALK

Synonyms: n/a

Product No.: 1369-0000-1

Lot: 003

Description: Human ALK G1202R, internal fragment, amino acids L_{1066}-S_{1437} (as in NCBI/Protein entry NP_004295.2), G1202R mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ALK G1202R Lot 001, was confirmed as ALK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 70,558 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

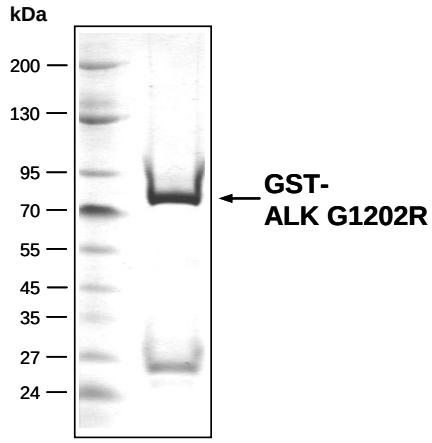
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

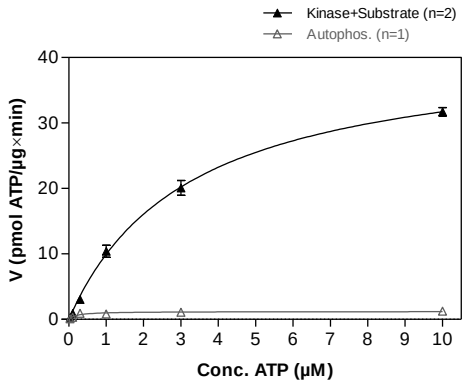
Biochemical Parameters:
Specific kinase activity (P_i transfer): 42 pmol/µg × min
ATP-K_M: 3.2 µM

ALK G1202R Lot 003:
Coomassie stain



3.0 µg GST-ALK G1202R

ALK G1202R Lot 003:
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: TRK-C derived peptide 80 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
ALK G1202R Lot 003 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

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ProQinase™ ALK G1202R

Product No.: 1369-0000-1

Recombinant Proteins

GST-ALK G1202R 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 KMFKDRLCHK 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 PRFILLELMA GRDLKSFLRE 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
 601 VPPLLVSQQA KREEERS                                              660
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: ALK fragment **boxed**: G1202R

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