

Certificate of Analysis



ALK R1275Q

anaplastic lymphoma kinase (Ki-1)

Recombinant Human Active Protein Kinase

HGNC Symbol: ALK

Synonyms: n/a

Product No.: 1181-0000-1

Lot: 001

Description: Human ALK R1275Q, internal fragment, amino acids L₁₀₆₆-S₁₄₃₇ (as in NCBI/Protein entry NP_004295.2), R1275Q mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ALK R1275Q Lot 001, was confirmed as ALK with a R1275Q mutation by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 70,431 Da

Expression: Baculovirus infected Sf9 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: 0.332 µg/µl

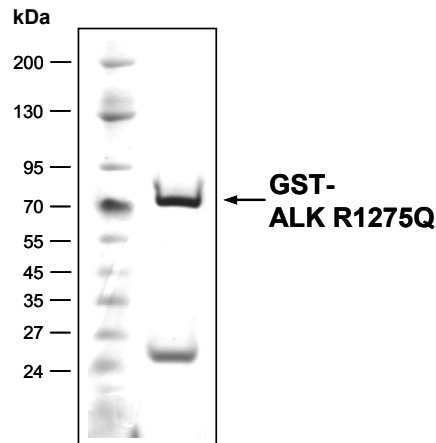
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 68 pmol/µg×min

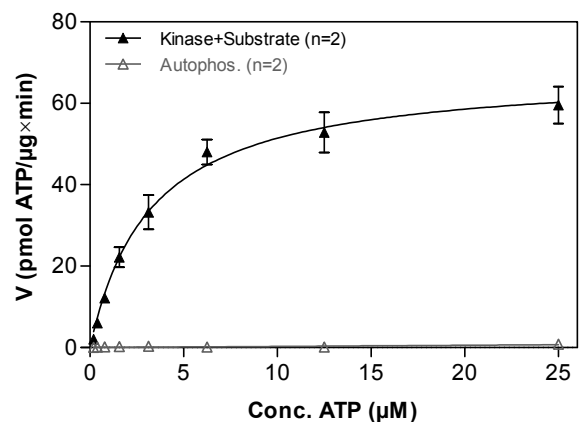
ATP-K_M: 3.2 µM

ALK R1275Q Lot 001: Coomassie stain



2.0 µg GST-ALK R1275Q

ALK R1275Q 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: TRK-C derived peptide 40 µg/ml
 - ALK R1275Q: 1.0 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: ALK R1275Q Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



This product was manufactured at ProQinase in Freiburg, Germany and is for in vitro research use only, not for use in humans or animals. ProQinase disclaims any warranty explicitly or implied that the use of the product or parts of the product is free from third party intellectual property claims unless this is explicitly stated.

CoA V8.1 ALK_R1275Q(GST-HIS-tag)_Lot001_V4.doc

ALK R1275Q

Product No.: 1181-0000-1

ALK R1275Q Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHG	RDS LEVLFOG	240
241	PLAMVLQAMQ	MELQSPEYKL	SKLRTSTIMT	DYNPNYCFAG	KTSSISDLKE	VPRKNITLIR	300
301	GLGHGAFGEV	YEQVSGMPN	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISKFNHQIV	360
361	RCIGVSLQSL	PRFILLELMA	GGDLKSFLRE	TRPRPSQPSS	LAMLDLLHVA	RDIACGCQYL	420
421	EENHFIHRDI	AARNCLLTCP	GPGRVAKIGD	FGMAQDIYRA	SYRKGGCAM	LPVKWMPPEA	480
481	FMEGIFTSKT	DTWSFGVLLW	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR	540
541	IMTQCWQHQP	EDRPNFAIIL	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG	600
600	VPPLLSVQQA	KREEERS					660

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:** ALK fragment **boxed:** R1275Q

ALK wt ¹ Amino Acid Sequence							
1	MGAIGLLWLL	PLLLSTAAVG	SGMGTGQRAG	SPAAGPPLQP	REPLSYSRLQ	RKSLAVDFVV	60
61	PSLFRVYARD	LLLPPSSSEL	KAGRPEARGS	LALDCAPLLR	LLGPAPGVSW	TAGSPAPAEA	120
121	RTLSRVLKG	SVRKLRRAKQ	LVLELGEEAI	LEGCVGPPGE	AAVGLLQFNL	SELSWWIRQ	180
181	GEGRLRIRLM	PEKKASEVGR	EGRLSAAIRA	SQPRLLFQIF	GTGHSSLESP	TNMPSPSPDY	240
241	FTWNLTWIMK	DSFPFLSHRS	RYGLECSFDF	PCELEYSPLP	HDLRNQSWSW	RRIPSEEASQ	300
301	MDLLDGPAG	RSKEMPRGSF	LLLNTSADSK	HTILSPWMRS	SSEHCTLAVS	VHRHLQPSGR	360
361	YIAQLLPHNE	AAREILLMPT	PGKHGWTVLQ	GRIGRPDNP	RVALEYISSG	NRSLAVDFF	420
421	ALKNCSEGT	PGSKMALQSS	FTCWNGTVLQ	LGQACDFHQD	CAQGEDESQM	CRKLPVGFYC	480
481	NFEDGFCGWT	QGTLSPTPQ	WQVRTLKDAR	FQDHQDHALL	LSTTDVPASE	SATVTSATFP	540
541	APIKSSPCEL	RMSWLIRGVL	RGNVSLVLE	NKTGKEQGRM	VWHVAAYEGL	SLWQWMVPLP	600
600	LDVSDRFWLQ	MVAWWGQGS	AIVAFDNISI	SLDCYLTISG	EDKILQNTAP	KSRNLFERNP	660
661	NKELKPGENS	PRQTPIFDPT	VHWFITTCGA	SGPHGPTQAQ	CNNAYQNSNL	SVEVGSEGPL	720
721	KGIQIWKVPA	TDTYSISGYG	AAGGKGGKNT	MMRSHGVSVL	GIFNLEKDDM	LYILVGQQGE	780
781	DACPSTNQLI	QKVCIGENNV	IEEEIRVNR	VHEWAGGGGG	GGGATYVFKM	KDGVVPLII	840
841	AAGGGGRAYG	AKTDTFHPER	LENNSSVLGL	NGNSGAAGGG	GGWNTSLL	WAGKSLQEGA	900
901	TGGHSCPQAM	KKGWETRGG	FGGGGGCCSS	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	YEQVSGMPN	1140
1141	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISKFNHQIV	RCIGVSLQSL	PRFILLELMA	1200
1201	GGDLKSFLRE	TRPRPSQPSS	LAMLDLLHVA	RDIACGCQYL	EENHFIHRDI	AARNCLLTCP	1260
1261	GPGRVAKIGD	FGMAQDIYRA	SYRKGGCAM	LPVKWMPPEA	FMEGIFTSKT	DTWSFGVLLW	1320
1321	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR	IMTQCWQHQP	EDRPNFAIIL	1380
1381	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG	VPPLLSVQQA	KREEERS	1440
1441	PPPLPTTSSG	KAACKPTAAE	ISVRVPRGPA	VEGGHVNMAF	SQSNPPSELH	KVHGSRNKPT	1500
1501	SLWNPTYGSW	FTEKPTKNN	PIAKKEPHDR	GNLGLEGSCT	VPPNVATGRL	PGASLLEPS	1560
1561	SLTANMKEVP	LFRLRHFCG	NVNYGYQQG	LPLEAATAPG	AGHYEDTILK	SKNSMNQPGP	1620

blue: ALK sequence expressed in fusionprotein **Red:** variant in fusionprotein

¹NCBI/Protein accession number NP_004295.2