

Certificate of Analysis



ALK (HIS-tag)

anaplastic lymphoma kinase (Ki-1)

Recombinant Human Active Protein Kinase

Synonyms: n/a

Product No.: 1048-0000-1

Lot: 005

Description: Human ALK, internal fragment, amino acids L₁₀₆₆-S₁₄₃₇ (as in GenBank entry NM_004304.3), N-terminal HIS₆ fusion protein, expressed in E.coli

Theoretical MW_{Fusion Protein}: 47,991 Da

Expression: E.coli

Purification: Immobilized Metal Affinity Chromatography

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

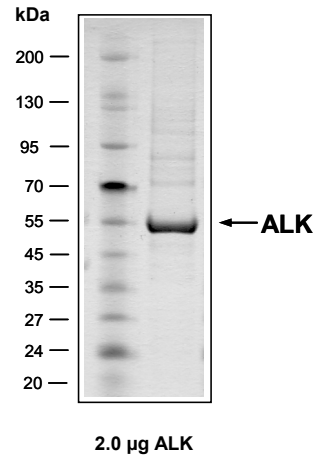
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.673 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

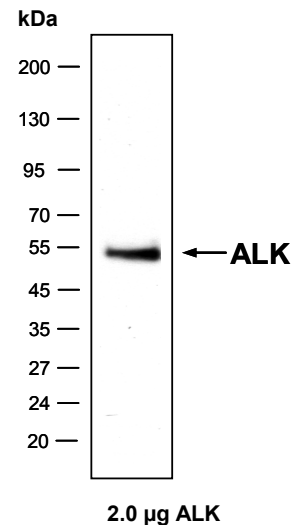
Determination of kinase activity:

- Assay conditions (25µl assay volume):
 - 70 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(Ala:Glu:Lys:Tyr)_{6:2:5:1} (Sigma P-1152), 1 µg/well
 - ALK: 50 ng/well
- ADP-Glo Assay (Promega)

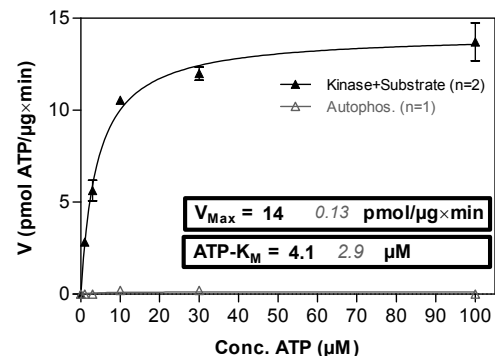
ALK Lot 005: Coomassie stain



ALK Lot 005: Western Blot analysis (anti penta-His)



ALK Lot 005: Kinase activity (Promega ADP-Glo® Assay)



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ALK Recombinant Fusion Protein Amino Acid Sequence											
1	MD	YKDDDDDKD	YKDDDDDKDYK	DDDDDKDYKDD	DDKSGGGS	LQ	AMQ	MELQ	SPE	YKLSKLRTST	60
61	IMTDYNPNYC	FAGKTSSISD	LKEVPRKNIT	LIRGLGHGAF	GEVYEQVSG	MPNDPSPLQV					120
121	AVKTLPEVCS	EQDELDLME	ALIISKFNHQ	NIVRCIGVSL	QSLPRFILE	LMAGGDLKSF					180
181	LRSTRPRPSQ	PSSLAMLDLL	HVARDIACGC	QYLEENHFIH	RDIAARNCLL	TCPGPGRVAK					240
241	IGDFGMARDI	YRASYYRKGG	CAMPLVKWMP	PEAFMEGIFT	SKTDTWSFGV	LLWEIFSLGY					300
301	MPYPSKSNQE	VLEFVTSGGR	MDPPKNCPPG	VYRIMTQCWQ	HQPEDRPNFA	IILERIEYCT					360
361	QDPDVINTAL	PIEYGPLVEE	EEKVPVRPKD	PEGVPPLLVS	QQA	KREEERS	KLLE	HHHHHH			420
421	HH										480

1-218: GST Green: FLAG Red: HIS6-tag blue: ALK fragment

ALK wt ORF (taken from GenBank entry NM_004304.3)												
1	MGAIGLLWLL	PLLLSTA AVG	SGMGTGQ RAG	SPAAGPPLQ P	REPLSYSRLQ	RKSLAVDFV V						60
61	PSLFRVYARD	LLLPPSSSEL	KAGRPEARGS	LALDCAPLLR	LLGPAPGVSW	TAGSPAPAEA						120
121	RTLSRVLKGG	SVRKLRRAKQ	LVLELGEEAI	LEGCVGPPGE	AAVGLLQFNL	SELFSSWIRQ						180
181	GEGRLRIRLM	PEKKASEVGR	EGRLSAAIRA	SQPRLLFQIF	GTGHSSLESP	TNMPSPSPDY						240
241	FTWNLTWIMK	DSFPFLSHRS	RYGLECSFDF	PCELEYS PPL	HDLRNQSWSW	RRIPSEEASQ						300
301	MDLLDGP GAE	RSKEMPRGSF	LLLNTSADSK	HTILSPWMRS	SSEHCTLAVS	VHRHLQPSGR						360
361	YIAQLLP HNE	AAREILLMPT	PGKHGWTVLQ	GRIGRPD NPF	RVALEYISSG	NRSLSAVDFF						420
421	ALKNCSEGT S	PGSKMALQSS	FTCWNGTVLQ	LGQACDFH QD	CAQGEDESQ M	CRKLPVGFYC						480
481	NFEDGFCGWT	QGTLSPHTPQ	WQVRTLK DAR	FQDHQDHALL	LSTTDVPASE	SATVTSATFP						540
541	APIKSSPCEL	RMSWLIRGVL	RGNVSLVLVE	NKTGKEQGRM	VWHVAAYEGL	SLWQWMVLPL						600
600	LDVSDRFLWQ	MVAWWGQGS R	AIVAFDNISI	SLDCYLTISG	EDKILQNTAP	KSRNLFERNP						660
661	NKELKPGENS	PRQTPIFDPT	VHWLFTTCGA	SGPHGPTQAQ	CNNAYQNSNL	SVEVGSEGPL						720
721	KGIQIWKVPA	TDTYSISGYG	AAGGKGKNT	MMRSHGVS VL	GIFNLEKDDM	LYILVGQQGE						780
781	DACPSTNQ LI	QKVCIGENNV	IEEEIRVNRS	VHEWAGGGGG	GGGATYVFKM	KDGPVPLII						840
841	AAGGGGRAYG	AKTDTFHPER	LENNSSVLGL	NGNSGAAGGG	GGWNDNTSL L	WAGKSLQEGA						900
901	TGGHSCPQAM	KKWGWETRGG	FGGGGGGCCS	GGGGGGYIGG	NAASNNDPEM	DGEDGVSFIS						960
961	PLGILYTPAL	KVMEGHGEVN	IKHYLNC SHC	EVDECHMDPE	SHKVICFCDH	GTVLAEDGVS						1020
1021	CIVSPTPEPH	LPLSLILSVV	TSALVAALVL	AFSGIMIVYR	RKHQELQAMQ	MELQSP EYKL						1080
1081	SKLRTSTIMT	DYNPNYCFAG	KTSSISDLKE	VPRKNITLIR	GLGHGAFGEV	YEGQVSGMPN						1140
1141	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISKFNHQNIV	RCIGVSLQSL	PRFILELMA						1200
1201	GGDLKSFLRE	TRPRPSQPSS	LAMLDLLHVA	RDIACGCQYL	EENHFIHRDI	AARNCLLTCP						1260
1261	GPGRVAKIGD	FGMARDIYRA	SYRKGGC CAM	LPVKWMPPEA	FMEGIFTSKT	DTWSFGVLLW						1320
1321	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR	IMTQCWQHQP	EDRPNFAIIL						1380
1381	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG	VPPLLVSQQA	KREEERS PAA						1440
1441	PPPLPTTSSG	KAACKPTAAE	ISVRVPRGPA	VEGGHV NMAF	SQSNPPSELH	KVHGSRNKPT						1500
1501	SLWNPTYGSW	FTEKPTKKNN	PIAKKEPHDR	GNLGLEGSCT	VPPNVATGRL	PGASLLLEPS						1560
1561	SLTANMKEVP	LFRLRHFCPG	NVNYGYQQQG	LPLEAATAPG	AGHYEDTILK	SKNSMNQPGP						1620

blue: fragment of ALK expressed in fusionprotein

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