

# Certificate of Analysis



## ALK F1174S

anaplastic lymphoma kinase (Ki-1)

Recombinant Human Active Protein Kinase

HGNC Symbol: ALK

Synonyms: n/a

Product No.: 1179-0000-1

Lot: 001

**Description:** Human ALK F1174S, internal fragment, amino acids L<sub>1066</sub>-S<sub>1437</sub> (as in NCBI/Protein entry NP\_004295.2), F1174S mutation, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** ALK F1174S Lot 001, was confirmed as ALK by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 70,399 Da

**Expression:** Baculovirus infected Sf9 cells

**Purification:** GST-Affinity Chromatography

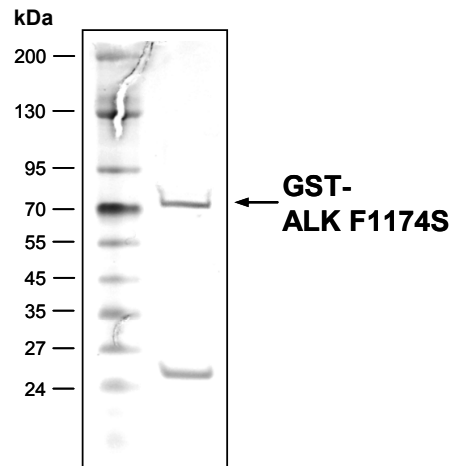
**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

**Protein concentration:** 0.203 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

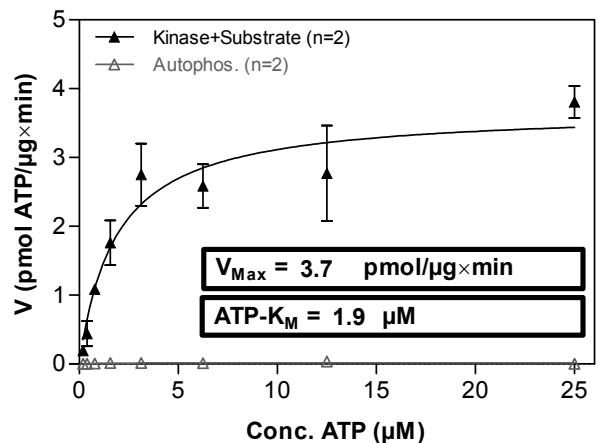
**Biochemical Parameters:**  
Specific activity: 4 pmol/µg×min  
ATP-K<sub>M</sub>: 1.9 µM

ALK F1174S Lot 001:  
Coomassie stain



2.0 µg GST-ALK F1174S

ALK F1174S Lot 001:  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: TRK-C derived peptide
  - ALK F1174S: 1.0 µg / ml
- Filter binding assay
  - MSFC membrane (Millipore)

**Additional assay technology:** ALK F1174S Lot 001

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

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# ALK F1174S

Product No.: 1179-0000-1

ALK F1174S 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	PMGHHHHHGG	RDSLEVLFGQ	240
241	PLAMVLOAMQ	MELQSPEYKL	SKLRTSTIMT	DYNPNYCFAG	KTSSISDLKE	VPRKNITLIR	300
301	GLGHGAFGEV	YEQVSGMPN	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISK <sup>S</sup> NHQNIV	360
361	RCIGVSLQSL	PRFILLELMA	GGDLKSFLRE	TRPRPSQPSS	LAMLDLLHVA	RDIACGCQYL	420
421	EENHFIHRDI	AARNCLLTC	GPGRVAKIGD	FGMARDIYRA	SYRKGGCAM	LPVKWMPPEA	480
481	FMEGIFTSKT	DTWSFGVLLW	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR	540
541	IMTQCWQHQP	EDRPNFMAIL	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG	600
600	VPPLLSVQQA	KREEERS					660

1-218: GST    **Red:** HIS6-tag    **Green:** 3C    **blue:** ALK fragment    **S:** F1174S

ALK wt <sup>1</sup> amino acid sequence							
1	MGAIGLLWLL	PLLLSTAAVG	SGMGTGQRAG	SPAAGPPLQP	REPLSYSRLQ	RKSLAVDFVV	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	PCELEYSPLP	HDLRNQSWSW	RRIPSEEASQ	300
301	MDLLDGPAGE	RSKEMPRGSF	LLLNTSADSK	HTILSPWMRS	SSEHCTLAVS	VHRHLQPSGR	360
361	YIAQLLPHNE	AAREILLMPT	PGKHGWTVLQ	GRIGRPDNP	RVALEYISSG	NRSLAVDFF	420
421	ALKNCSEGST	PGSKMALQSS	FTCWNGTVLQ	LGQACDFHQD	CAQGEDESQM	CRKLPVGFYC	480
481	NFEDGFCGWT	QGTLSPTPQ	WQVRTLKDAR	FQDHQDHALL	LSTTDVPASE	SATVTSATFP	540
541	APIKSSPCEL	RMSWLIRGVL	RGNVSLVLE	NKTGKEQGRM	VWHVAAYEGL	SLWQWMVPLP	600
600	LDVSDRFLWQ	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	IEEEIRVNRS	VHEWAGGGGG	GGGATYVFKM	KDGPVPLII	840
841	AAGGGGRAYG	AKTDTFHPER	LENNSSVLGL	NGNSGAAGGG	GGWNDNTSL	WAGKSLQEGA	900
901	TGGHSCPQAM	KKGWETRGG	FGGGGGGCS	GGGGGGYIGG	NAASNNDPEM	DGEDGVSFIS	960
961	PLGILYTPAL	KVMEGHGEVN	IKHYLNCSHC	EVDECHMDPE	SHKVICFCDH	GTVLAEDGVS	1020
1021	CIVSPTPEPH	LPLSLILSV	TSALVAALVL	AFSGIMIVYR	RKHQELQAMQ	MELQSPEYKL	1080
1081	SKLRTSTIMT	DYNPNYCFAG	KTSSISDLKE	VPRKNITLIR	GLGHGAFGEV	YEQVSGMPN	1140
1141	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISK <sup>S</sup> NHQNIV	RCIGVSLQSL	PRFILLELMA	1200
1201	GGDLKSFLRE	TRPRPSQPSS	LAMLDLLHVA	RDIACGCQYL	EENHFIHRDI	AARNCLLTC	1260
1261	GPGRVAKIGD	FGMARDIYRA	SYRKGGCAM	LPVKWMPPEA	FMEGIFTSKT	DTWSFGVLLW	1320
1321	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR	IMTQCWQHQP	EDRPNFMAIL	1380
1381	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG	VPPLLSVQQA	KREEERS	1440
1441	PPPLPTTSSG	KAACKPTAAE	ISVRVPRGPA	VEGGHVNMAF	SQSNPPSELH	KVHGSRNKPT	1500
1501	SLWNPTYGSW	FTEKPTKKN	PIAKKEPHDR	GNLGLEGSCT	VPPNVATGRL	PGASLLEPS	1560
1561	SLTANMKEVP	LFRLRHFCPG	NVNYGYQQG	LPLEAATAPG	AGHYEDTILK	SKNSMNQPGP	1620

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

<sup>1</sup>NCBI/Protein accession number NP\_004295.2