Certificate of Analysis

REACTION BIOLOGY

ProQinase™ EML4 ALK F1174L

Echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase fusionprotein

Recombinant Human Active Protein Kinase

HGNC Symbol: n/a

Synonyms: n/a

Product No.: 1247-0000-1

Lot: 003

Description: Human pathological fusionprotein EML4 ALK, full length, amino acids M_1-P_{1059} (as in NCBI/Protein entry BAF73611.1) with a F1174L mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: EML4 ALK F1174L Lot 003, was confirmed as EML4 ALK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 145.413 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

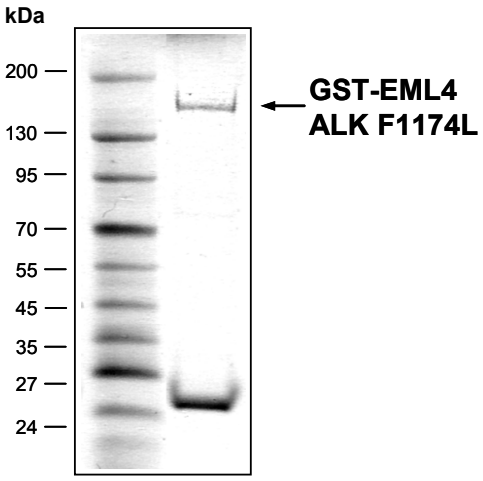
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

Protein concentration: 0.111 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

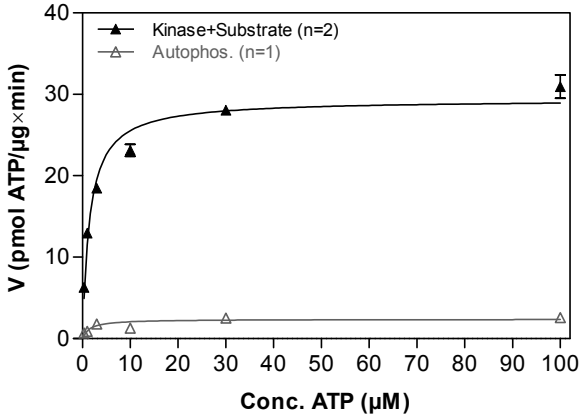
Biochemical Parameters:
Specific activity: 29 pmol/µg×min
ATP-K_M: 1.5 µM

EML4 ALK F1174L Lot 003:
Coomassie stain



2.0 µg GST-EML4 ALK F1174L

EML4 ALK F1174LLot 003:
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
  EML4 ALK F1174L: 2.0 µg / ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology: EML4 ALK F1174L Lot 003 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

Promega

Recombinant Proteins

REACTION BIOLOGY

ProQinase™ EML4 ALK F1174L

Product No.: 1247-0000-1

Recombinant Proteins

EML4 ALK F1174L 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 PLAMDGFAGS LDDSISAAST SDVQDRLSAL ESRVQQQEDE ITVLKAALAD VLRRLAISED  300
 301 HVASVKKSVS SKGQPSPRAV IPMSCITNGS GANRKPSHTS AVSIAGKETL SSAAKSGTEK  360
 361 KKEKPQGQRE KKEESHSNDQ SPQIRASPSP QPSSQPLQIH RQTPESKNAT PTKSIKRPSP  420
 421 AEKSHNSWEN SDDSRNKLSK IPSTPKLIPK VTKTADKHKD VIINQEGEYI KMFMRGRPIT  480
 481 MFIPSDVDNY DDIRTELPPE KLKLEWAYGY RGKDCRANVY LLPTGEIVYF IASVVVLFNY  540
 541 EERTQRHYLG HTDCVKCLAI HPDKIRIATG QIAGVDKDGR PLQPHVRVWD SVTLSTLQII  600
 601 GLGTFERGVG CLDFSKADSG VHLCVIDDSN EHMLTVWDWQ RKAKGAEIKT TNEVVLAVEF  660
 661 HPTDANTIIT CGKSHIFFWT WSGNSLTRKQ GIFGKYEKPK FVQCLAFLGN GDVLTGDSGG  720
 721 VMLIWSKTTV EPTPGKGPKV YRRKHQELQA MQMELQSPEY KLSKLRTSTI MTDYNPNYCF  780
 781 AGKTSSISDL KEVPRKNITL IRGLGHGAFG EVYEGQVSGM PNDPSPLQVA VKTLPEVCSE  840
 841 QDELDFLMEA LIISKLNHQN IVRCIGVSLQ SLPRFILLEL MAGGDLKSFL RETRPRPSQP  900
 901 SSLAMLDLLH VARDIACGCQ YLEENHFIHR DIAARNCLLT CPGPGRVAKI GDFGMARDIY  960
 961 RASYYRKGGC AMLPVKWMPP EAFMEGIFTS KTDTWSFGVL LWEIFSLGYM PYPSKSNQEV 1020
1021 LEFVTSGGRM DPPKNCPGPV YRIMTQCWQH QPEDRPNFAI ILERIEYCTQ DPDVINTALP 1080
1081 IEYGPLVEEE EKVPVRPKDP EGVPPLLVSQ QAKREEERSP AAPPPLPTTS SGKAAKKPTA 1140
1141 AEVSVRVPRG PAVEGGHVNM AFSQSNPPSE LHRVHGSRNK PTSLWNPTYG SWFTEKPTKK 1200
1201 NNPIAKKEPH ERGNLGLEGS CTVPPNVATG RLPGASLLLE PSSLTANMKE VPLFRLRHFP 1260
1261 CGNVNYGYQQ QGLPLEAATA PGAGHYEDTI LKSKNSMNQP GP                    1320
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1-218: GST **Red: HIS6-tag** **Green: 3C** **blue:EML4ALK** **L**:F1174L ALK mutation

EML4 ALK wt[1] amino acid sequence

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   1 MDGFAGSLDD SISAASTSDV QDRLSALESR VQQQEDEITV LKAALADVLR RLAISEDHVA   60
  61 SVKKSVSSKG QPSPRAVIPM SCITNGSGAN RKPSHTSAVS IAGKETLSSA AKSGTEKKKE  120
 121 KPQGQREKKE ESHSNDQSPQ IRASPSPQPS SQPLQIHRQT PESKNATPTK SIKRPSPAEK  180
 181 SHNSWENSDD SRNKLSKIPS TPKLIPKVTK TADKHKDVII NQEGEYIKMF MRGRPITMFI  240
 241 PSDVDNYDDI RTELPPEKLK LEWAYGYRGK DCRANVYLLP TGEIVYFIAS VVVLFNYEER  300
 301 TQRHYLGHTD CVKCLAIHPD KIRIATGQIA GVDKDGRPLQ PHVRVWDSVT LSTLQIIGLG  360
 361 TFERGVGCLD FSKADSGVHL CVIDDSNEHM LTVWDWQKKA KGAEIKTTNE VVLAVEFHPT  420
 421 DANTIITCGK SHIFFWTWSG NSLTRKQGIF GKYEKPKFVQ CLAFLGNGDV LTGDSGGVML  480
 481 IWSKTTVEPT PGKGPKVYRR KHQELQAMQM ELQSPEYKLS KLRTSTIMTD YNPNYCFAGK  540
 541 TSSISDLKEV PRKNITLIRG LGHGAFGEVY EGQVSGMPND PSPLQVAVKT LPEVCSEQDE  600
 601 LDFLMEALII SKFNHQNIVR CIGVSLQSLP RFILLELMAG GDLKSFLRET RPRPSQPSSL  660
 661 AMLDLLHVAR DIACGCQYLE ENHFIHRDIA ARNCLLTCPG PGRVAKIGDF GMARDIYRAS  720
 721 YYRKGGCAML PVKWMPPEAF MEGIFTSKTD TWSFGVLLWE IFSLGYMPYP SKSNQEVLEF  780
 781 VTSGGRMDPP KNCPGPVYRI MTQCWQHQPE DRPNFAIILE RIEYCTQDPD VINTALPIEY  840
 841 GPLVEEEEKV PVRPKDPEGV PPLLVSQQAK REEERSPAAP PPLPTTSSGK AAKKPTAAEV  900
 901 SVRVPRGPAV EGGHVNMAFS QSNPPSELHR VHGSRNKPTS LWNPTYGSWF TEKPTKKNNP  960
 961 IAKKEPHERG NLGLEGSCTV PPNVATGRLP GASLLLEPSS LTANMKEVPL FRLRHFPCGN 1020
1021 VNYGYQQQGL PLEAATAPGA GHYEDTILKS KNSMNQPGP                        1080
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bold letters: expressed part of EML4 (**blue**) and ALK (**green**) **RED** letters: variant in Fusionprotein

[1]NCBI/Protein accession number BAF73611.1