

ProQinase™ NPM1 ALK F1174L

Nucleophosmin anaplastic lymphoma kinase fusionprotein

Recombinant Human Active Protein Kinase

HGNC Symbol: *n/a*

Synonyms: *n/a*

Product No.: 1281-0000-1

Lot: 005

Description: Human pathological fusionprotein NPM1 ALK, full length, amino acids M₁-P₆₈₀ (as in NCBI/Protein entry AAA58698.1) with a F1174L mutation, untagged, expressed in Sf9 insect cells

Product identity: NPM1 ALK F1174L Lot 005, was confirmed as NPM1 ALK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 75,661 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography, followed by 3C mediated removal of the GST tag

Activation: This kinase was not activated by special procedures

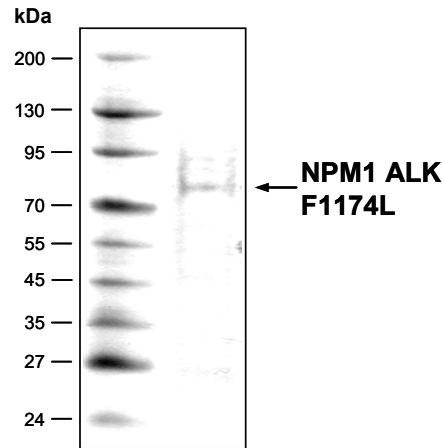
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20% glycerol

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

Protein concentration: 0.151 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

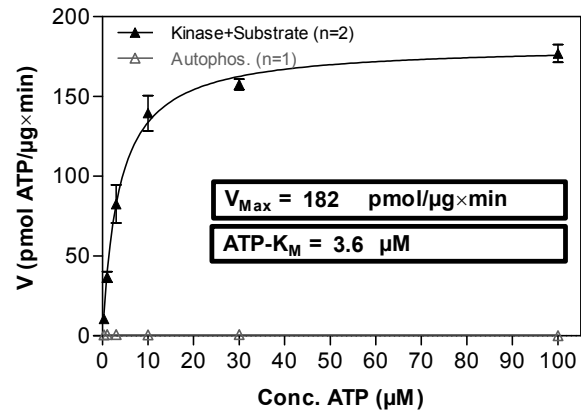
Biochemical Parameters:
Specific activity: 182 pmol/µg×min
ATP-K_M: 3.6 µM

NPM1 ALK F1174L Lot 005:
Coomassie stain



2.0 µg NPM1 ALK F1174L

NPM1 ALK F1174L Lot 005:
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
 - NPM1 ALK F1174L: 1.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: NPM1 ALK F1174L Lot 005 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



Recombinant Proteins

ProQinase™ NPM1 ALK F1174L

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NPM1 ALK F1174L Recombinant Fusion Protein Amino Acid Sequence									
1	GPL	AMEDSMD	MDMSPLRPQ	YLF	GCELKAD	KDYHFKVDND	ENEHQLSLRT	VSLGAGAKDE	60
61	LHIVEAEAMN	YEGSPIKVTL	ATLKMSVQPT	VSLGGFEITP	PVVLRLKCGS	GPVHISGQHL			120
121	VVYRRKHQEL	QAMQMELOSP	EYKLSKLR	TST	IMTDYNPNY	CFAGKTSSIS	DLKEVPRKNI		180
181	TLIRGLGHGA	FGEVYEQVS	GMPNDPSPLQ	VAVKTLPEVC	SEQDELDFLM	EALIISKLNH			240
241	QNIVRCIGVS	LQSLPRFILL	ELMAGGDLKS	FLRETRRPRS	QPSSLAMLDL	LHVARDIACG			300
301	CQYLEENHFI	HRDIAARNCL	LTCPGPGRVA	KIGDFGMARD	IYRASYYRKG	GCAMPLPVKWM			360
361	PPEAFMEGIF	TSKTDTWSFG	VLLWEIFSLG	YMPYPSKSNQ	EVLEFVTSGG	RMDPPKNC	PG		420
421	PVYRIMTQCW	QHQPEDRPNF	AIILERIEYC	TQDPDVINTA	LPIEYGPLVE	EEKVPVVRPK			480
481	DPEGVPPLLV	SQQAQREER	SPAAPPPLPT	TSSGKAAKPP	TAAEVSVRVP	RGPVAVGGHV			540
541	NMAFSQSNPP	SELHVVHGSR	NKPTSLWNPT	YGSWFTEKPT	KKNNPIAKKE	PHVRGNLGLLE			600
601	GSCTVPPNVA	TGRLPGASLL	LEPSSLTANM	KEVPLFRLRH	FPCGNVNYGY	QQQGLPLEAA			660
661	TAPGAGHYED	TILKSKNSMN	QPGP						720

1-4: legacy of 3C cleavage blue:NPM1ALK L: F1174L mutation boxed: SNP variation

NPM1 ALK wt ¹ amino acid sequence									
1	MEDSMDMDMS	PLRPQNYLFG	CELKADKDYH	FKVDNDENEH	QLSLRTVSLG	AGAKDELHIV		60	
61	EAEAMNYEGS	PIKVTLATLK	MSVQPTVSLG	GFEITPPVVL	RLKCGSGPVH	ISGQHLVVYR		120	
121	RKHQELQAMQ	MELQSPEYKL	SKLRTSTIMT	DYNPNYCFAG	KTSSISDLKE	VPRKNITLIR		180	
181	GLGHGAFGEV	YEQVSGMPN	DPSPLQVAVK	TLPEVCSEQD	ELDFLMEALI	ISKFNHQNIV		240	
241	RCIGVSLQSL	PRFILLELMA	GGDLKSFLRE	TRRPRSQPSS	LAMLDLLHVA	RDIACGCQYL		300	
301	EENHFIHRDI	AARNCLLTC	GPGRVAKIGD	FGMARDIYRA	SYRKGGCAM	LPVKWMPPEA		360	
361	FMEGIFTSKT	DTWSFGVLLW	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR		420	
421	IMTQCWQHQP	EDRPNFAIL	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG		480	
481	VPELLVSQQA	KREERSPAA	PPPLPTTSSG	KAAKPTAAE	VSVRVP	RGPA	VEGGHV	NMAF	540
541	SQSNPPSELH	KVHGSRNKPT	SLWNPTYGSW	FTEKPTKKN	PIAKKEPHDR	GNLGLEGSCT			600
601	VPPNVATGRL	PGASLLLEPS	SLTANMKEVP	LFRLRHFP	CG	NVNYGYQQQG	LPLEAATAPG		660
661	AGHYEDTILK	SKNSMNQPGP							720

bold letters: expressed part of NPM1 (blue) and ALK (green) RED letters: variant in Fusionprotein

¹NCBI/Protein accession number AAA58698.1

K551R and D589E: SNP variations see NCBI/dbSNP IDs: rs1881420, rs1881421

Recombinant Proteins