

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

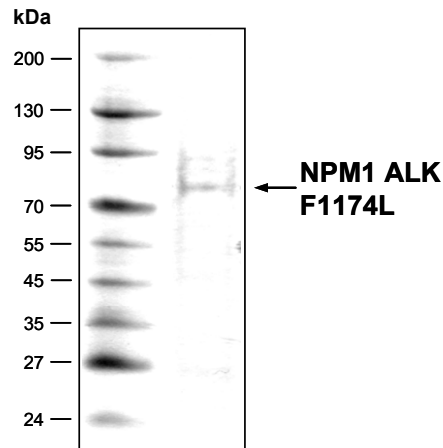
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

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

Biochemical Parameters:

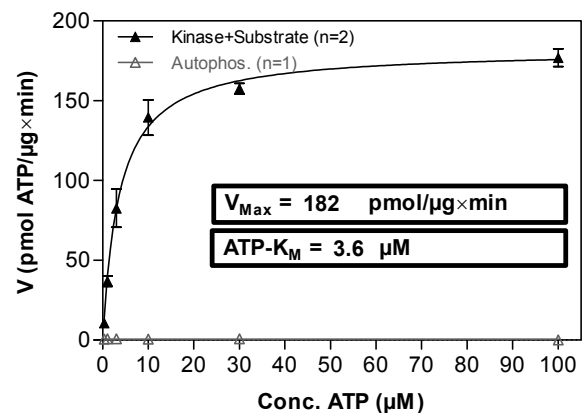
Specific activity: 182 pmol/µg×min
ATP-K_M: 3.6 µM

**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 ProQinase for the use with the ADP-Glo™ Kinase assay from 

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NPM1 ALK F1174L Recombinant Fusion Protein Amino Acid Sequence								
1	GPLA	MEDSMD	MDMSPLRPQN	YLFGCELKAD	KDYHFKVDND	ENEHQLSLRT	VSLGAGAKDE	60
61	LHIVEAEAMN	YEGSPIKVTL	ATLKMSVQPT	VSLGGFEITP	PVVLRLKCGS	GPVHISGQHL		120
121	VVYRRKHQEL	QAMQELQSP	EYKLSKLRTS	TIMTDYNPNY	CFAGKTSSIS	DLKEVPRKNI		180
181	TLIRGLGHGA	FGEVYEGQVS	GMPNDPSPLQ	VAVKTLPEVC	SEQDELDFLM	EALIISKI	NH	240
241	QNIVRCIGVS	LQSLPRFILL	ELMAGGDLKS	FLRETRPRPS	QPSSLAMLDL	LHVARDIACG		300
301	CQYLEENHFI	HRDIAARNCL	LTCPGPGRVA	KIGDFGMARD	IYRASYYRKG	GCAMLVPKWM		360
361	PPEAFMEGIF	TSKTDTWSTFG	VLLWEIFSLG	YMPYPSKSNQ	EVLEFVTSGG	RMDPPKNC	PG	420
421	PVYRIMTQCW	QHQPEDRPNF	AIILERIEYC	TQDPDVINTA	LPIEYGPLVE	EEEKVPVRPK		480
481	DPEGVPPLL	V	SQQA	KREEER	SPAAPPPLPT	TSSGKA	AKKP	540
541	NMAFSQSNPP	SELH	KVH	SRNKPT	SLWNPT	YGSWFTEKPT	KKNNPIAKKE	600
601	GSCTVPPNVA	TGRLPGASLL	LEPSSLTANM	KEVPLFRLRH	FPCGNVNYGY	QQQGLPLEAA		660
661	TAPGAGHYED	TILKSKNSMN	QPGP					720

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

NPM1 ALK wt ¹ amino acid sequence								
1	MEDSMD	MDMS	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	TRPRPSQPSS	LAMLDLLHVA	RDIAACGQYL		300
301	EENHFHHRDI	AARNCLLTCP	GPGRVAKIGD	FGMARDIYRA	SYRKGKGCAM	LPVKWMPPEA		360
361	FMEGIFTSKT	DTWSFGVLLW	EIFSLGYMPY	PSKSNQEVLE	FVTSGGRMDP	PKNCPGPVYR		420
421	IMTQCWQHQP	EDRPNFAIIL	ERIEYCTQDP	DVINTALPIE	YGPLVEEEEEK	VPVRPKDPEG		480
481	VPPLLVSQQA	KREEERS	PAA	PPPLPTTSSG	KA	AKKPTAAE	VSVRVPRGPA	540
541	SQSNPPSELH	KVH	SRNKPT	SLWNPTYGSW	FTEKPTKKNN	PIAKKEPHDR	GNLGLEGSCT	600
601	VPPNVATGRL	PGASLLLEPS	SLTANMKEVP	LFRLRHFP	CG	NVNYGYQQQG	LPLEAATAPG	660
661	AGHYEDTILK	SKNSMN	QPGP					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