Certificate of Analysis

REACTION BIOLOGY

ProQinase™ NPM1ALK

Nucleophosmin anaplastic lymphoma kinase fusionprotein

Recombinant Human Active Protein Kinase

HGNC Symbol: n/a

Synonyms: n/a

Product No.: 1236-0000-1

Lot: 005

Description: Human pathological fusionprotein NPM1ALK, full length, amino acids M_1-P_{680} (as in NCBI/Protein entry AAA58698.1), untagged, expressed in Sf9 insect cells

Product identity: NPM1ALK Lot 005, was confirmed as NPM1ALK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 75,695 Da

Expression host: Sf9 insect cells

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

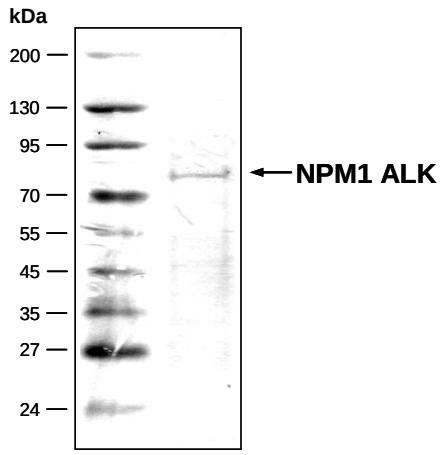
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20% glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.189 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

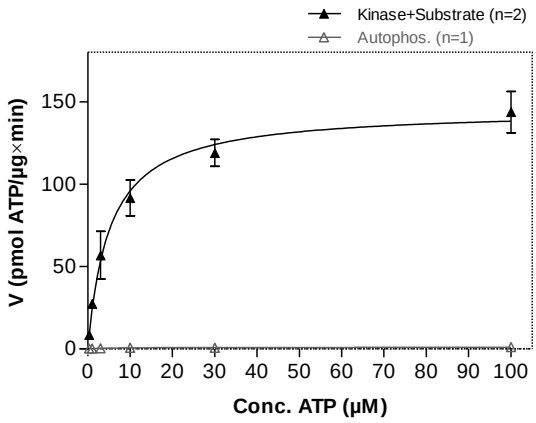
Biochemical Parameters:
Specific kinase activity (P_i transfer): 145 pmol/µg×min
ATP-K_M: 5.2 µM

NPM1ALK Lot 005:
Coomassie stain



2.0 µg NPM1 ALK

NPM1ALK 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 $MgCl_2$
  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
  NPM1 ALK: 1.0 µg / ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology: NPM1ALK Lot 005 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

Recombinant Proteins

ProQinase™ NPM1ALK

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NPM1ALK Recombinant Fusion Protein Amino Acid Sequence

1	GPLAMEDSMD MDMSPLRPQN YLFGCELKAD KDYHFKVDND ENEHQLSLRT VSLGAGAKDE	60
61	LHIVEAEAMN YEGSPIKVTL ATLKMSVQPT VSLGGFEITP PVVLRLKCGS GPVHISGQHL	120
121	VVYRRKHQEL QAMQMELQSP EYKLSKLRTS TIMTDYNPNY CFAGKTSSIS DLKEVPRKNI	180
181	TLIRGLGHGA FGEVYEGQVS GMPNDPSPLQ VAVKTLPEVC SEQDELDFLM EALIISKFNH	240
241	QNIVRCIGVS LQSLPRFILL ELMAGGDLKS FLRETRPRPS QPSSLAMLDL LHVARDIACG	300
301	CQYLEENHFI HRDIAARNCL LTCPGPGRVA KIGDFGMARD IYRASYYRKG GCAMLPVKWM	360
361	PPEAFMEGIF TSKTDTWSFG VLLWEIFSLG YMPYPSKSNQ EVLEFVTSGG RMDPPKNCPG	420
421	PVYRIMTQCW QHQPEDRPNF AIILERIEYC TQDPDVINTA LPIEYGPLVE EEEKVPVRPK	480
481	DPEGVPPLLV SQQAKREEER SPAAPPPLPT TSSGKAAKKP TAAEVSVRVP RGPAVEGGHV	540
541	NMAFSQSNPP SELHRVHGSR NKPTSLWNPT YGSWFTEKPT KKNNPIAKKE PHERGNLGLE	600
601	GSCTVPPNVA TGRLPGASLL LEPSSLTANM KEVPLFRLRH FPCGNVNYGY QQQGLPLEAA	660
661	TAPGAGHYED TILKSKNSMN QPGP	720

1-4: legacy of 3C cleavage **blue**: NPM1ALK **boxed**: variation from RefSeq

NPM1ALK 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 YEGQVSGMPN DPSPLQVAVK TLPEVCSEQD ELDFLMEALI ISKFNHQNIV	240
241	RCIGVSLQSL PRFILLELMA GGDLKSFLRE TRPRPSQPSS LAMLDLLHVA RDIACGCQYL	300
301	EENHFIHRDI AARNCLLTCP GPGRVAKIGD FGMARDIYRA SYYRKGGCAM LPVKWMPPEA	360
361	FMEGIFTSKT DTWSFGVLLW EIFSLGYMPY PSKSNQEVLE FVTSGGRMDP PKNCPGPVYR	420
421	IMTQCWQHQP EDRPNFAIIL ERIEYCTQDP DVINTALPIE YGPLVEEEEK VPVRPKDPEG	480
481	VPPLLVSQQA KREEERSPAA PPPLPTTSSG KAAKKPTAAE VSVRVPRGPA VEGGHVNMAF	540
541	SQSNPPSELH KVHGSRNKPT SLWNPTYGSW FTEKPTKKNN PIAKKEPHDR GNLGLEGSCT	600
601	VPPNVATGRL PGASLLLEPS SLTANMKEVP LFRLRHFPCG NVNYGYQQQG LPLEAATAPG	660
661	AGHYEDTILK SKNSMNQPGP	720

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

[1]NCBI/Protein accession number AAA58698.1
K551R and D589E: SNP variations see NCBI/dbSNP IDs: rs1881420, rs1881421

Recombinant Proteins