

Certificate of Analysis

ALK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-954, 14-954-K, 14-954M

Parent Lot # D14KP005N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human, ALK1 amino acids 142-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 86% by SDS-PAGE and Coomassie blue staining. MW = 45 kDa.

Specific Activity (Parent lot# D14KP005N): 338U/mg, where one unit of ALK1, active activity is defined as 1nmol phosphate incorporated into casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.472mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

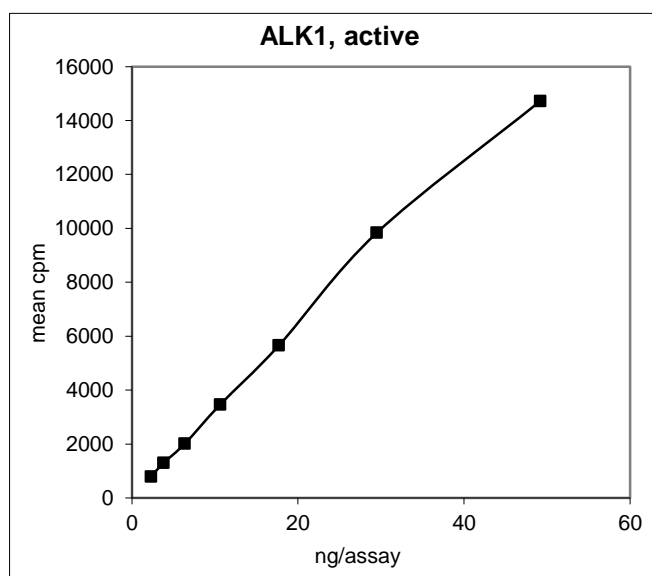
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

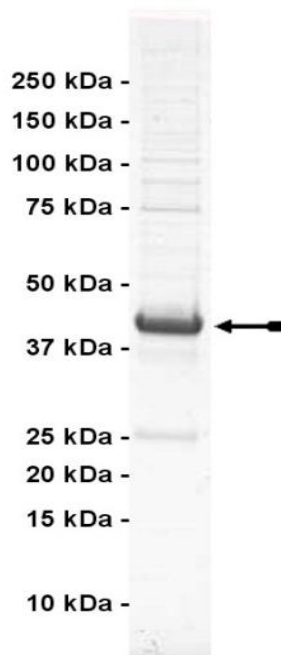
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2-49ng of this lot of enzyme phosphorylated casein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as ALK1 with the translated sequence listed on page three



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of ALK1, active

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Kinase Assay Protocol

Stock Solutions:

- 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- Casein:** Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
- Manganese Chloride:** Use at a final assay concentration of 2.5mM. Prepare a 100mM solution and add 0.625µl per assay point.
- Sodium Chloride:** Use at a final assay concentration of 20mM. Prepare a 3M stock and add 0.1667µl per assay point.
- ALK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–49ng per assay point.
- [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

- Add 5µl of 5 x reaction buffer per assay to wells.
- Add 4.208µl of dH₂O.
- Add 0.167µL of 3M sodium chloride to wells
- Add 0.625µL 100mM manganese chloride to wells
- Add 2.5µl of casein to wells.
- Add **2.5µl (2–49ng) ALK1, active.**
- Add 10µl of diluted [γ -³³P]ATP mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5µl of 3% phosphoric acid.
- Transfer a 10µl aliquot onto the appropriate area of a P30 Filtermat.
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
- Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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ALK1 Sequence Information

Protein	human ALK1
Tags	N-terminal 6His
Native sequence	H31 of the recombinant protein is equivalent to H142 of human ALK1
Accession number	GenBank L17075.1

Recombinant ALK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF HVRRRQEKQR GLHSELGESS LILKASEQGD
61 SMLGDLDDSD CTTGSGSGLP FLVQRTVARQ VALVECVGKG RYGEVWRGLW HGESVAVKIF
121 SSRDEQSWFR ETEIYNTVLL RHDNILGFIA SDMTSRNSST QLWLIITHYHE HGSLYDFLQR
181 QTLEPHLALR LAVSAACGLA HLHVEIFGTQ GKPAIAHRDF KSRNVLVKSN LQCCIADLGL
241 AVMHSQGS DY LDIGNNPRVG TKRYMAPEVL DEQIRTDCFE SYKWTDIWAF GLVLWEIARR
301 TIVNGIVEDY RPPFYDVVPN DPSFEDMKKV VCVDQQTPTI PNRLAADPVL SGLAQMMREC
361 WYPNPSARLT ALRIKKTLOK ISNSPEKPKV IQ
  
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Recombinant ALK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc catgtccgac ggaggcagga gaagcagcgt
121 ggccctgcaca gcgagctggg agagtccagt ctcacctga aagcatctga gcagggcgac
181 agcatggttg gggacctcct ggacagtgac tgcaccacag ggagtggctc agggctcccc
241 ttcttggtgc agaggacagt ggcacggcag gttgccttg tggagtgtgt gggaaaaggc
301 cgctatggcg aagtgtggcg gggcttgtg cacggtgaga gtgtggccgt caagatcttc
361 tcctcgaggg atgaacagtc ctggttccgg gagactgaga tctataacac agtgttgctc
421 agacacgaca acatcctagg ctctacgcc tcagacatga cctcccgaa ctcgagcacg
481 cagctgtggc tcatcacgca ctaccacgag cacggctccc tctacgactt tctgcagaga
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1141 attagcaaca gtccagagaa gcctaaagtg attcaatag
  
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