

Certificate of Analysis

ALK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-614, 14-614-K, 14-614M

Parent Lot # 25467U

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 GST-tagged, recombinant, human ALK4 amino acids 150–end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 92% by SDS-PAGE and Coomassie blue staining. MW = 63.8kDa.

Specific Activity (Parent Lot# 25467U): 38U/mg, where one unit of ALK4 activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.28mg/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.

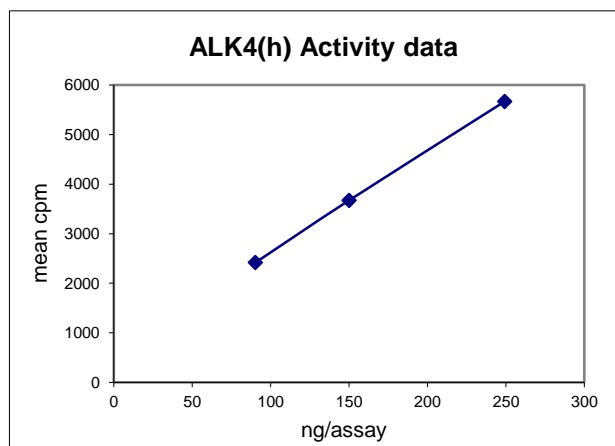
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap

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.

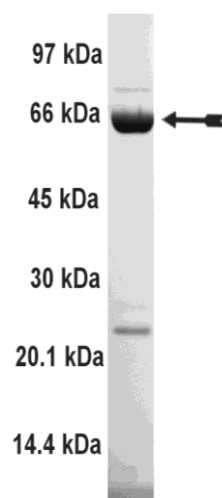
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 57–228ng of this lot of enzyme phosphorylated 2 mg/ml 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 product identity as ALK4 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active ALK4.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Casein:** Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
- 3. ALK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 57–228ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[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):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **casein**.
3. Add **2.5µl (57–228ng) ALK4, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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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ALK4 Sequence Information

<u>Protein</u>	human ALK4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	N231 of recombinant sequence is equivalent to N150 of native human ALK4
<u>Accession number</u>	GenBank NM_004302

Recombinant ALK4 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP EF  NYHQRVYHNR
241  QRLDMEDPSC  EMCLSKDKTL  QDLVYDLSTS  GSGSGLPLFV  QRTVARTIVL  QEIIIGKGRFG
301  EVWRGRWRGG  DVAVKIFSSR  EERSWFREAE  IYQTVMLRHE  NILGFIAADN  KDNGTWTQLW
361  LVSDYHEHGS  LFDYLNRYTV  TIEGMIKLAL  SAASGLAHLH  MEIVGTQGKP  GIAHRDLKSK
421  NILVKKNGMC  AIADLGLAVR  HDAVTDTIDI  APNQRVGTRK  YMAPEVLDET  INMKHFDSFK
481  CADIYALGLV  YWEIARRCNS  GGVHEEYQLP  YYDLVPSDPS  IEEMRKVVCD  QKLRPNIPNW
541  WQSYEALRVM  GKMMRECWYA  NGAARLTALR  IKKTLSQLSV  QEDVKI
  
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Recombinant ALK4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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1741  caggaagacg  tgaagatcta  a
  
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