

## Certificate of Analysis

### ALK6, active

#### (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-941, 14-941-K, 14-941M

Parent Lot # D13NP012N

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 ALK6 amino acids 148-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 89% by SDS-PAGE and Coomassie blue staining. MW = 44kDa.

**Specific Activity (Parent lot# D13NP012N):** 159U/mg, where one unit of ALK6, active activity is defined as 1nmol phosphate incorporated into casein per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.531mg/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.

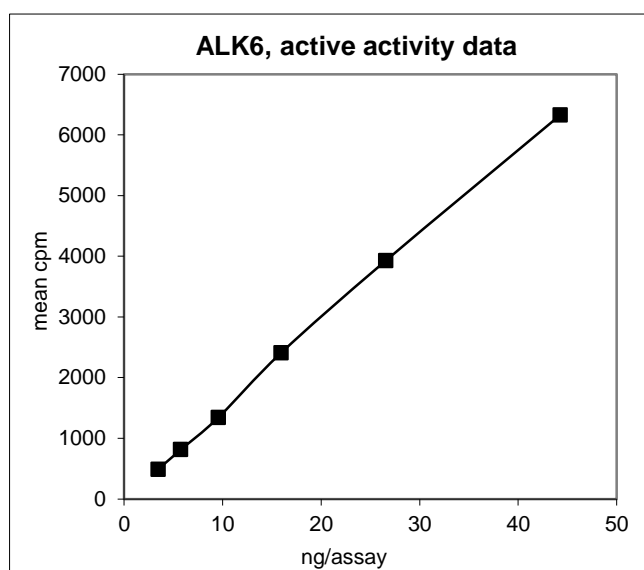
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

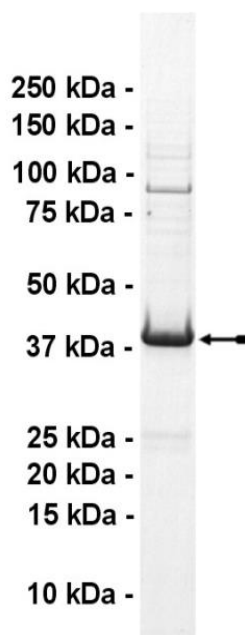
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 3–44ng 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 ALK6 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 ALK6, 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 1mM. Prepare a 100mM solution and add 0.25µl per assay point.
- Sodium Chloride:** Use at a final assay concentration of 20mM. Prepare a 3M stock and add 0.1667µl per assay point.
- ALK6, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3–44ng per assay point.
- [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]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 4.583µl of dH<sub>2</sub>O.
- Add 0.167µL of 3M sodium chloride to wells
- Add 0.25µL 100mM manganese chloride to wells
- Add 2.5µl of casein to wells.
- Add **2.5µl (3–44ng) ALK6, active.**
- Add 10µl of diluted [ $\gamma$ -<sup>33</sup>P]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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## ALK6 Sequence Information

<b><u>Protein</u></b>	human ALK6
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	F30 of the recombinant protein is equivalent to F148 of human ALK6
<b><u>Accession number</u></b>	GenBank NM_001203

### **Recombinant ALK6 amino acid sequence:**

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF RYKRQETRPR YSIGLEQDET YIPPGESLRD
61 LIEQSQSSGS GSGLLLVQR TIAKQIQMVK QIGKGRYGEV WMGKWRGEKV AVKVFVFTTEE
121 ASWFRETEIY QTVLMRHENI LGFIAADIKG TGSWTQLYLI TDYHENGSLY DYLKSTTLDA
181 KSMLKLAYSS VSGLCHLHTE IFSTQGKPAI AHRDLKSKNI LVKKNGTCCI ADLGLAVKFI
241 SDTNEVDIPP NTRVGTKRYM PPEVLDES LNHNHFQSYIMA DMYSFGLILW EVARRCVSGG
301 IVEEYQLPYH DLVSPDPSYE DMREIVCIKK LRPSFPNRWS SDECLRQMGK LMTECWAHNP
361 ASRLTALRVK KTLAKMSESQ DIKL
  
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### **Recombinant ALK6 nucleotide sequence:**

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc cggataaaa gacaagaaac cagacctcga
121 tacagcattg ggtagaaca ggatgaaact tacattcctc ctggagaatc cctgagagac
181 ttaattgagc agtctcagag ctcaggaagt ggatcaggcc tccctctgct ggtccaaagg
241 actatagcta agcagattca gatggtgaaa cagattggaa aaggctcgta tggggaagt
301 tggatgggaa agtggcgtgg cgaaaaggta gctgtgaaag tgttcttcac cacagaggaa
361 gccagctggg tcagagagac agaaatatac cagacagtgt tgatgaggca tgaaacatt
421 ttgggtttca ttgctgcaga tatcaaaggg acagggtcct ggaccagtt gtacctaatc
481 acagactatc atgaaaatgg ttccctttat gattatctga agtccaccac cctagacgct
541 aatcaatgc tgaagttagc ctactcttct gtcagtggct tatgtcattt acacacagaa
601 atcttttagta ctcaaggcaa accagcaatt gccatcgag atctgaaaag taaaacatt
661 ctggtgaaga aaaatggaac ttgctgtatt gctgacctgg gcctggctgt taaatttatt
721 agtgatacaa atgaagttga cataccacct aacctcgag ttggcaccaa acgctatatg
781 cctccagaag tgttgacga gagcttgaac agaaatcact tccagtctta catcatggct
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1021 agtgatgagt gtctaaggca gatgggaaaa ctcatgacag aatgctgggc tcacaatcct
1081 gcatcaaggc tgacagccct gcgggttaag aaaacactg caaaatgtc agagtcaccg
1141 gacattaac tctaa
  
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