

Certificate of Analysis

ALK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-937, 14-937-K, 14-937M

Parent Lot # D13HP016N

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 ALK2 amino acids 147-end, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 91% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

Formulation: 0.834mg/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.

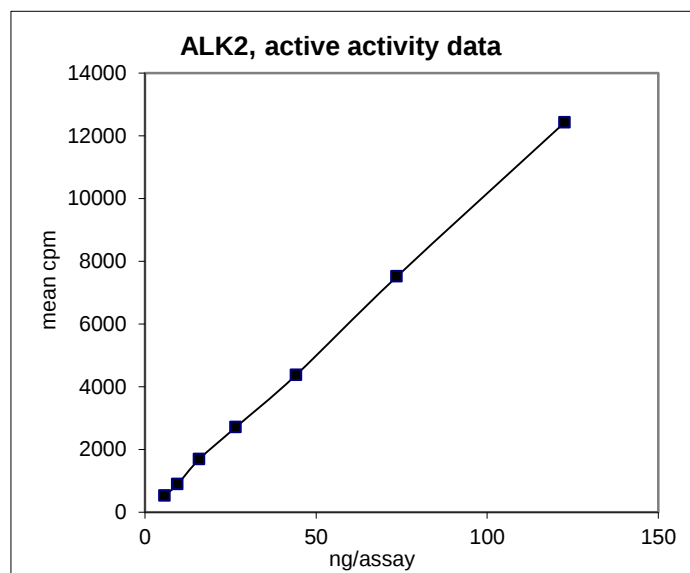
Specific Activity (Parent lot# D13HP016N): 134U/mg, where one unit of ALK2, active activity is defined as 1nmol phosphate incorporated into casein per minute at 30°C with a final ATP concentration of 100µM.

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.

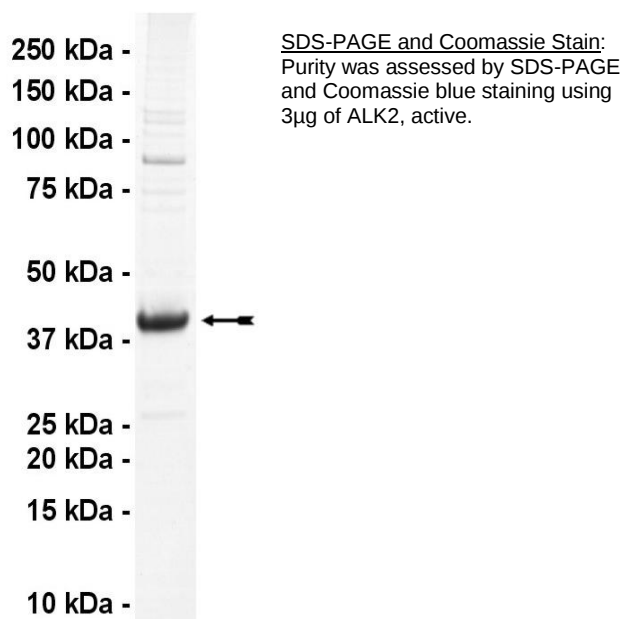
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6–123g 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 ALK2 with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 200mM HEPES pH7.4.
2. **Casein:** Use at a final assay concentration of 1mg/ml. Prepare a 20mg/ml stock and add 1.25µl of stock per assay point.
3. **Manganese Chloride:** Use at a final assay concentration of 2.5mM. Prepare a 100mM solution and add 0.625µl per assay point.
4. **Sodium Chloride:** Use at a final assay concentration of 20mM. Prepare a 3M stock and add 0.1667µl per assay point.
5. **ALK2, active:** Dilute with 200mM HEPES pH7.4, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–123ng per assay point.
6. **[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):

1. Add 5µl of 5 x reaction buffer per assay to wells
2. Add 5.46µl water per assay point to wells
3. Add 0.1667µl sodium chloride
4. Add 0.625µl manganese chloride
5. Add **2.5µl (6–123ng) ALK2, active.**
6. Add 1.25µl of casein.
7. Add 10µl of diluted [γ-³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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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ALK2 Sequence Information

<u>Protein</u>	human ALK2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	R31 of the recombinant protein is equivalent to R147 of human ALK2
<u>Accession number</u>	GenBank NM_001105

Recombinant ALK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF RKFKRRNQER LNPRDVEYGT IEG LITTNVG
61 DSTLADLLDH SCTSGSGSGL PFLVQRTVAR QITLLECVGK GRYGEVWRGS WQGENVAVKI
121 FSSRDEKSWF RETELYNTVM LRHENILGFI ASDMTSRHSS TQLWLITHYH EMGSLYDYLQ
181 LTTLDTVSCL RIVLSIASGL AHLHIEIFGT QGKPAIAHRD LKSKNILVKK NGQCCIADLG
241 LAVMHSQSTN QLDVGNNPRV GTKRYMAPEV LDETIQVDCF DSYKRVDIWA FGLVLWEVAR
301 RMVSNQIVED YKPPFYDVVP NDPSFEDMRK VVCVDQQRPN IPNRWFSDPT LTSLAKLMKE
361 CWYQNPSARL TALRIKKTLT KIDNSLDKLG TDC

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Recombinant ALK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgcatgga tccggaattc cgaaaattta aaaggcgcaa ccaagaacgc
121 ctcaatcccc gagacgtgga gtatggcact atcgaagggc tcatcaccac caatgttgga
181 gacagcactt tagcagattt attggatcat tcgtgtacat caggaagtgg ctctggctct
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