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Certificate of Analysis

Alk, active

(Recombinant enzyme expressed in Sf21 cells)
Item # 14-555, 14-555-K, 14-555M
Parent Lot # WAB0375

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 ALK, amino acids 1058–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 59% by SDS-PAGE and Coomassie blue staining. MW = 63.8kDa.

Specific Activity (Parent lot# WAB0375): 254U/mg, where one unit of ALK activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.51mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

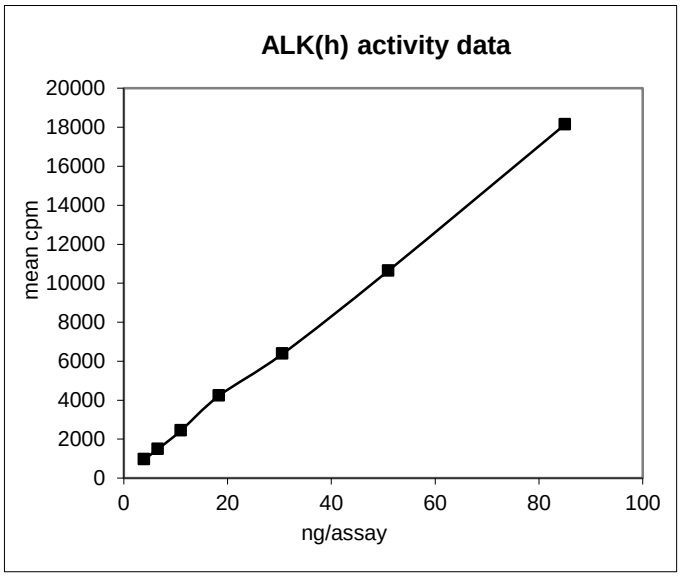
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

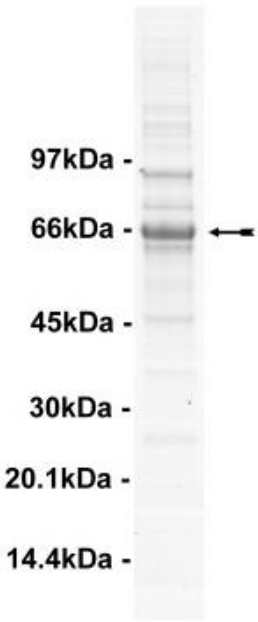
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4–85ng of this lot of enzyme phosphorylated 250µM (KKKSPGEYVNIEFG) 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 ALK 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 ALK, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH 7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Make a 2.5mM stock. Use 2.5µl of stock per assay point.
3. **ALK, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–85ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 2.5mM **(KKKSPGEYVNIEFG)**.
3. Add **2.5µl (4–85ng) ALK, active**.
4. Add 5µl dH_20.
5. Add 10µl of diluted [γ-^{33}P] 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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ALK Sequence Information

Protein human ALK

Tags *N*-terminal 6His

Native sequence V10 of recombinant sequence is equivalent to V1058 of human ALK

Accession number GenBank U62540

Recombinant ALK amino acid sequence:

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   1 MHHHHHHEFV YRRKHQELQA MQMELQSPEY KLSKLRTSTI MTDYNPNYCF AGKTSSISDL
  61 KEVPRKNITL IRGLGHGAFG EVYEGQVSGM PNDPSPLQVA VKTLPEVCSE QDELDFLMEA
 121 LIISKFNHQN IVRCIGVSLQ SLPRFILLEL MAGGDLKSFL RETRPRPSQP SSLAMLDLLH
 181 VARDIACGCQ YLEENHFIHR DIAARNCLLT CPGPGRVAKI GDFGMARDIY RASYYRKGGC
 241 AMLPVKWMPP EAFMEGIFTS KTDTWSFGVL LWEIFSLGYM PYPSKSNQEV LEFVTSGGRM
 301 DPPKNCPGPV YRIMTQCWQH QPEDRPNFAI ILERIEYCTQ DPDVINTALP IEYGPLVEEE
 361 EKVPVRPKDP EGVPPLLVSQ QAKREEERSP AAPPPLPTTS SGKAAKKPTA AEVSVRVPRG
 421 PAVEGGHVNM AFSQSNPPSE LHKVHGSRNK PTSLWNPTYG SWFTEKPTKK NNPIAKKEPH
 481 DRGNLGLEGS CTVPPNVATG RLPGASLLLE PSSLTANMKE VPLFRLRHFP CGNVNYGYQQ
 541 QGLPLEAATA PGAGHYEDTI LKSKNSMNQP GP
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Recombinant ALK nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcgtg taccgccgga agcaccagga gctgcaagcc
  61 atgcagatgg agctgcagag ccctgagtac aagctgagca agctccgcac ctcgaccatc
 121 atgaccgact acaaccccaa ctactgcttt gctggcaaga cctcctccat cagtgacctg
 181 aaggaggtgc cgcggaaaaa catcaccctc attcggggtc tgggccatgg cgcctttggg
 241 gaggtgtatg aaggccaggt gtccggaatg cccaacgacc caagccccct gcaagtggct
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 361 ctgatcatca gcaaattcaa ccaccagaac attgttcgct gcattggggt gagcctgcaa
 421 tccctgcccc ggttcatcct gctggagctc atggcggggg gagacctcaa gtccttcctc
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1621 cagggcttgc ccttagaagc cgctactgcc cctggagctg gtcattacga ggataccatt
1681 ctgaaaagca agaatagcat gaaccagcct gggccctga
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