

Certificate of Analysis

IKK α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-461, 14-461-K, 14-461M

Parent Lot # WAA0185

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, full-length human IKK alpha expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 89.8% by SDS-PAGE and Coomassie blue staining. MW = 112kDa.

Specific Activity (Parent lot# WAA0185): 106U/mg, where one unit of IKK alpha activity is defined as 1nmol phosphate incorporated into $200\mu\text{M}$ (KKKKERLLDDRHSGLDSMKDEE) per minute at 30°C with a final ATP concentration of 100 μM .

Formulation: 0.253mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

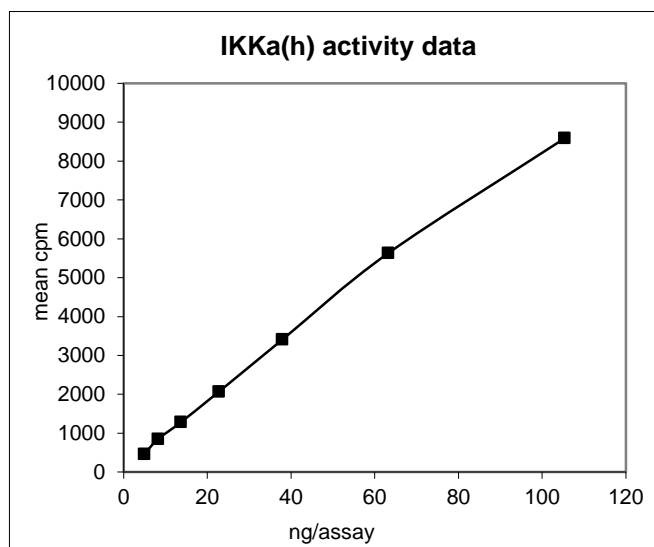
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

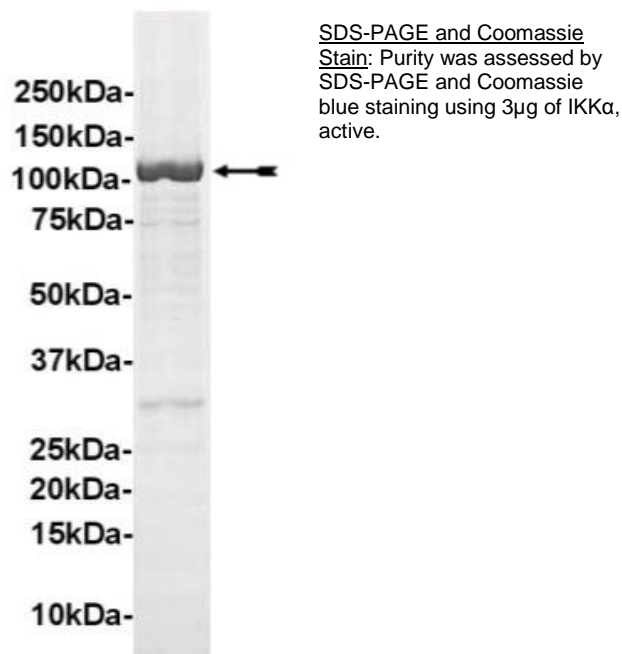
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5–105ng of this lot of enzyme phosphorylated $200\mu\text{M}$ (KKKKERLLDDRHSGLDSMKDEE) 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 IKK α with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKKKERLLDDRHSGLDSMKDEE):** Use at a final assay concentration of 200 μ M. Prepare a 1mM stock and add 5 μ l of stock to each assay point.
3. **IKK α active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–105ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 5 μ l of **(KKKKERLLDDRHSGLDSMKDEE)**.
3. Add **2.5 μ l (5–105ng) IKK α , active**.
4. Add 2.5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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IKKα Sequence Information

<u>Protein</u>	human IKKα
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M231 of the recombinant protein is equivalent to M1 of human IKKα
<u>Accession number</u>	GenBank AF012890

Recombinant IKKα amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL  LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPFE MERPPGLRPG
241 AGGPWEMRER LGTGGFGNVC LYQHRELDL IAIKSCRLEL STKNRERWCH EIQIMKKLNH
301 ANVVKACDVP EELNILIHDV PLLAMEYCSG GDLRKL LNKP ENCCGLKESQ ILSLLSDIGS
361 GIRYLHENKI IHRDLKPENI VLQDVGGKII HKIIDLGYAK DVDQGSLSCTS FVGTLLQYLAP
421 ELFENKPYTA TVDYWSFGTM VFECIAGYRP FLHHLQPFTW HEKIKKKDKP CIFACEEMSG
481 EVRFSSHLPQ PNSLCSLIVE PMENWLQML NWDPPQRRGGP VDLTLKQPRC FVLMDHILNL
541 KIVHILNMTS AKIISFLLPP DESLHSLQSR IERETGINTG SQELLSETGI SLDPRKPAASQ
601 CVLDGVRGCD SYMVYLFDKS KTVYEGPFAS RSLSDCVNYI VQDSKIQLPI IQLRKVWAEA
661 VHYVSGLKED YSRLFQQRRA AMLSLLRYNA NLTKMKNTLI SASQQLKAKL EFFHKSIQLD
721 LERYSEQMTY GISSEKMLKA WKEMEKAH YAEVGVIGYL EDQIMSLHAE IMELQKSPYG
781 RRQGDLMESL EQRAIDLKQ LKHRPSDHSY SDSTEMVKII VHTVQSQDRV LKELFGHLSK
841 LLGCKQKIID LLPKVEVALS NIKEADNTVM FMQGKRQKEI WHLLKIACTQ SSARSLVGSS
901 LEGAVTPQTS AWPPTSAEH DHSLSCVVTP QDGETSAQMI EENLNCLGHL STIIHEANEE
961 QGNSMMNLDW SWLTE
    
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Recombinant IKKα nucleotide sequence:

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\      1  atgtccccta tactaggta ttgaaaatt aaggccttg tgcaaccac tcgacttctt
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121 tggcgaaaca aaaagttaga attgggttg gagtttcca atcttcctta ttatattgat
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1321 gtatttgaat gtattgctgg atatagcct tttttgcatc atctgcagc atttacctgg
1381 catgagaaga ttaagaagaa ggatccaaa tgtatatttg catgtgaaga gatgtcagga
    
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2881 cagggaata gtatgatgaa tcttgattgg agttgggttaa cagaatga

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