

## Certificate of Analysis

### IKK $\epsilon$ , active

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

Item # 14-926, 14-926-K, 14-926M

Parent Lot # D12NP008N

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 FLAG and GST-tagged, recombinant, human IKK $\epsilon$  full length, expressed by baculovirus in Sf21 insect cells. Purified by immunoaffinity chromatography. Purity 84.7% by SDS-PAGE and Coomassie blue staining. MW = 109kDa.

**Specific Activity (Parent lot# D12NP008N):** 805U/mg, where one unit of IKK $\epsilon$ , active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 0.142mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10mM  $\beta$ -glycerophosphate, 1mM sodium orthovanadate, 1mM NaF, 28% v/v glycerol, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

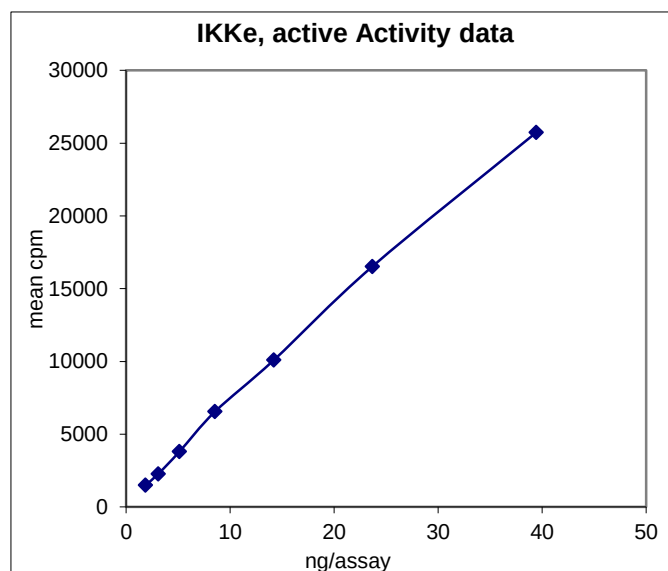
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

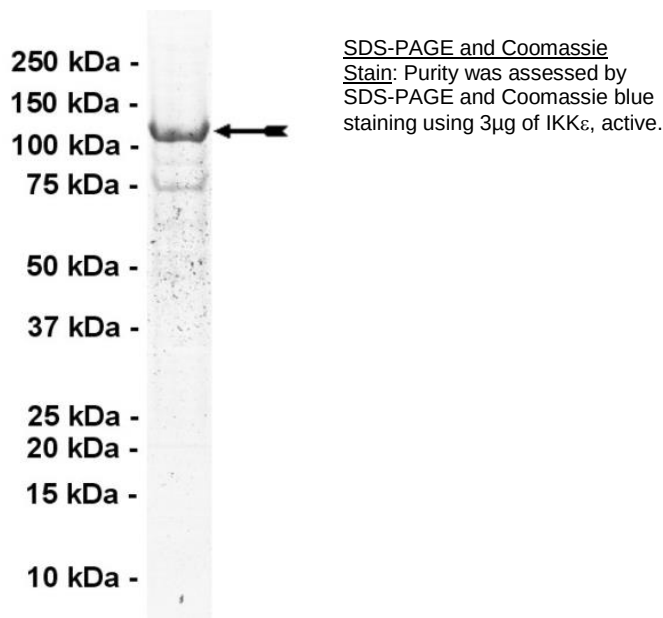
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 2–39ng of this lot of enzyme phosphorylated 2mg/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 identity as IKK $\epsilon$  with the translated 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. **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. **IKK $\epsilon$ , active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–39ng per assay point.
4. **[ $\gamma$ -<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):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **casein**.
3. Add **2.5µl (2–39ng) IKK $\epsilon$ , active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>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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## IKK $\epsilon$ Sequence Information

|                                |                                                                             |
|--------------------------------|-----------------------------------------------------------------------------|
| <b><u>Protein</u></b>          | human IKK $\epsilon$                                                        |
| <b><u>Tags</u></b>             | N-terminal FLAG and N-terminal GST                                          |
| <b><u>Native sequence</u></b>  | M247 of the recombinant protein is equivalent to M1 of human IKK $\epsilon$ |
| <b><u>Accession number</u></b> | GenBank NM_014002                                                           |

### Recombinant IKK $\epsilon$ amino acid sequence:

```

1 MDYKDDDDKS PILGYWKIKG LVQPTRLLE YLEEKYEHL YERDEGDKWR NKKFELGLEF
61 PNLPHYIDGD VKLTQSMAL RYIADKHNML GGCPKERA EI SMLEGA VLDI RYGVSR IAYS
121 KDFETLKVD F LSKLPEMLKM FEDRLCHKTY LNGDHVTHPD FMLYDALDVV LYMDPMCLDA
181 FPKLVCFKKR IEAIPQIDKY LKSSKYIAWP LQGWQATFGG GDHPPKSDLV PRGSHNQTSL
241 YKKAGTMQST ANYLWHTDDL LGQGATASVY KARNKKS GEL VAVKVFN TTS YLRPREVQVR
301 EFVLRKLNH QNIVKLF AVE ETGGS RQKVL VMEYC SSGSL LSVLESP ENA FGLPEDEF LV
361 VLRCVVAGMN HLRENGIVHR DIKPGNIMRL VGEEGQSIYK LTDFGAAREL DDDEKFVSVY
421 GTEEYLHPDM YERAVLRKPQ QKAFGVTVDL WSIGVTLYHA ATGSLPFIPF GGPRRNKEIM
481 YRITTEKPAG AIAGAQRREN GPLEWSY TLP ITCQLSLGLQ SQLVPILANI LEVEQAKCWG
541 FDQFFAETSD ILQRVVVHVF SLSQAVLHHI YIHAHNTIAI FQEAVHKQTS VAPRHQEYLF
601 EGHLCVLEPS VSAQHIAHTT ASSPLT LFT AIPKGLAFRD PALDVPKFVP KVDLQADYNT
661 AKGVLGAGYQ ALRLARALLD GQELMFRGLH WMEVLQATC RRTLEVARTS LLYLSSSLGT
721 ERFSSVAGTP EIQLKAAAE LRSRLRTLA E VLSRCSQ NIT ETQESLSSLN RELVKS RDQV
781 HEDRSIQQIQ CCLDKMNF IY KQFKKSRMRP GLGYNEEQIH KLDKVNFSHL AKRLLQVFQE
841 ECVQKYQASL VTHGKRMRV HETRNLRLV GCSVAACNTE AQGVQESLSK LLEELSHQLL
901 QDRAKGAQAS PPPIAPY PSP TRKDLLLHMQ ELCEGMKLLA SDLLDNNRII ERLNRVPAPP
961 DV

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### Recombinant IKK $\epsilon$ nucleotide sequence:

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1 atggactaca aagacgatga cgataaatcc cctatactag gttattggaa aattaagggc
61 cttgtgcaac ccactcgact tcttttggaa tatcttgaag aaaaatatga agagcatttg
121 tatgagcgcg atgaaggtga taaatggcga aacaaaaagt ttgaattggg tttggagttt
181 cccaatcttc cttattatat tgatggtgat gttaaattaa cacagtctat ggccatcata
241 cgttatatag ctgacaagca caacatgttg gttggtgttc caaaagagcg tgcagagatt
301 tcaatgcttg aaggagcggg tttggatatt agatacggtg tttcgagaat tgcatatagt
361 aaagactttg aaactctcaa agttgatttt ctagcaagc tacctgaaat gctgaaaatg
421 ttcgaagatc gtttatgtca taaaacatat ttaaatggtg atcatgtaac ccatcctgac
481 ttcatgttgt atgacgctct tgatgttgtt ttatacatgg acccaatgtg cctggatgcg
541 ttcccaaaat tagtttgttt taaaaaacgt attgaagcta tcccacaaat tgataagtac
601 ttgaaatcca gcaagtatat agcatggcct ttgcagggct ggcaagccac gtttgggtgt
661 ggcgaccatc ctccaaaatc ggatctggtt ccgctggat ccacaaacca gacgtcgcta
721 tacaaaaaag ccggcacgat gcagagcaca gccattacc tgtggcacac agatgacctg
781 ctggggcagg gggccactgc cagtgtgtac aaggcccga acaagaaatc cggagagctg
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901 gagtttgagg tcctgcggaa gctgaaccac cagaacatcg tcaagctctt tgcggtggag
961 gagacgggcg gaagccggca gaaggtactg gtgatggagt actgctccag tgggagcctg
1021 ctgagtgtgc tggagagccc tgagaatgcc tttgggctgc ctgaggatga gttcctgggtg
1081 gtgctgcgct gtgtggtggc cggcatgaac cacctgcggg agaacggcat tgtgcatcgc
1141 gacatacaagc cggggaacat catgcgcctc gtaggggagg aggggcagag catctacaag
1201 ctgacagact tcggcgctgc ccgggagctg gatgatgatg agaagttcgt ctcggtctat
1261 gggactgagg agtacctgca tcccagatg tatgagcggg cggtgcttcg aaagccccag

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1321 caaaaagcgt tcggggtgac tgtggatctc tggagcattg gaggtagcctt gtaccatgca
1381 gccactggca gcctgccctt catccccttt ggtggggccac ggagggaacaa ggagatcatg
1441 taccggatca ccacagagaa gccggctggg gccattgcag gtgcccagag gcgggagaac
1501 gggcccctgg agtggagcta caccctcccc atcacctgcc agctgtcact ggggctgcag
1561 agccagctgg tgcccatcct ggccaacatc ctggagggtg agcaggccaa gtgctggggc
1621 ttcgaccagt tctttgcgga gaccagtgc atcctgcagc gaggttgtcgt ccatgtcttc
1681 tccctgtccc aggagtcctt gcaccacatc tatatccatg cccacaacac gatagccatt
1741 ttccaggagg ccgtgcacaa gcagaccagt gtggccccc gacaccagga gtacctcttt
1801 gagggtcacc tctgtgtcct cgagcccagc gtctcagcac agcacatcgc ccacacgacg
1861 gcaagcagcc ccctgaccct cttcagcaca gccatcccta aggggctggc cttcagggac
1921 cctgctctgg acgtcccaa gtctgtcccc aaagtggacc tgcaggcgga ttacaacact
1981 gccaaaggcg tgttggggcg cggtaccag gccctgcggc tggcacgggc cctgctggat
2041 gggcaggagc taatgtttcg ggggctgcac tgggtcatgg aggtgctcca ggccacatgc
2101 agacggactc tggaagtggc aaggacatcc ctctctacc tcagcagcag cctgggaact
2161 gagaggttca gcagcgtggc tggaacgcct gagatccagg aactgaaggc ggctgcagaa
2221 ctgaggtcca ggctgcggac tctagcggag gtctctcca gatgctcca aaatatcacg
2281 gagaccagg agagcctgag cagcctgaac cgggagctgg tgaagagccg ggatcaggta
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2401 aaacagttca agaagtctag gatgaggcca gggcttggct acaacgagga gcagattcac
2461 aagctggata aggtgaattt cagtcattta gccaaaagac tcctgcaggt gttccaggag
2521 gaggcgtgc agaagtatca agcgtcctta gtcacacacg gcaagaggat gagggtgggtg
2581 cacgagacca ggaaccacct gcgcctggtt ggctgttctg tggctgcctg taacacagaa
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2701 caggaccgag caaagggggc tcaggcctcg ccgcctccca tagctcctta cccagccct
2761 acacgaaagg acctgcttct ccacatgcaa gagctctgcg aggggatgaa gctgctggca
2821 tctgacctcc tggacaacaa ccgcatcatc gaacggctaa atagagtccc agcacctcct
2881 gatgtctga

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