

Certificate of Analysis

IKK ϵ , 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 ϵ 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.

Formulation: 0.142mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10mM β -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.

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

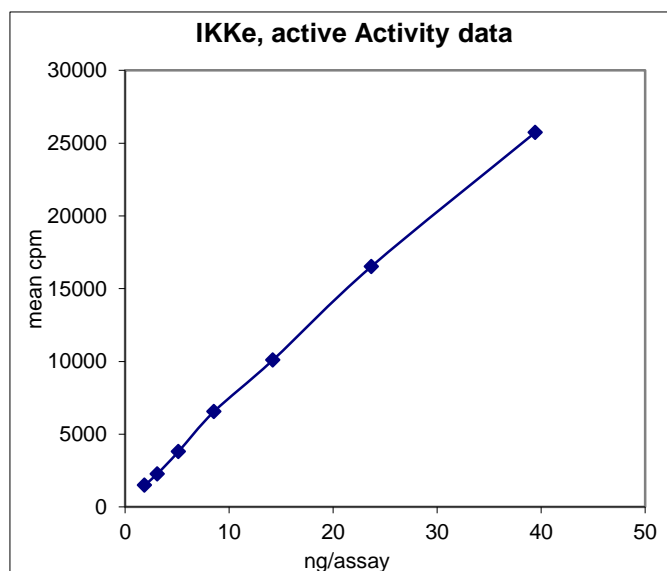
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

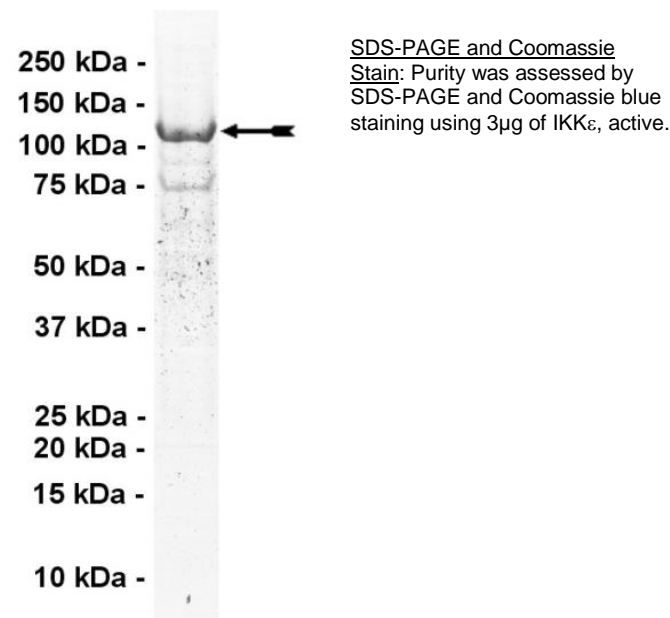
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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 ϵ 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. IKKε,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. [γ-³³P]ATP:** 2.5 x MgAc/[γ³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ³³P]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) IKKε, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]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 ϵ Sequence Information

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

Recombinant IKK ϵ amino acid sequence:

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1 MDYKDDDDKS PILGYWKIKG LVQPTRLLE YLEEKYEHL YERDEGDKWR NKKFELGLEF
61 PNLPHYIDGD VKLTQSMAL I RYIADKHNML GGCPKERA EI SMLEGA VLDI RYGVSR IAYS
121 KDFETLKVDF LSKLPEMLKM FEDRLCHKTY LNGDHVTHPD FMLYDALDVV LYMDPMCLDA
181 FPKLVCFKKR IEAIPQIDKY LKSSKYIAWP LQGWAQTFGG GDHPPKSDLV PRGSHNQTSL
241 YKKAGTMQST ANYLWHTDDL LGQGATASVY KARNKKSSEL VAVKVFNTTS YLRPREVQVR
301 EFEVLRKLNH QNIVKLF AVE ETGGSRQKVL VMEYCSSGSL LSVLESPENA FGLPEDEFLV
361 VLRCVVAGMN HLRENGIVHR DIKPGNIMRL VGEEGQSIYK LTDFGAAREL DDDEK FVSVY
421 GTEEYLHPDM YERAVLRKPQ QKAFGVTVDL WSIGVTLYHA ATGSLPFIPF GGPRRNKEIM
481 YRITTEKPA G AIAGAQRREN GPLEWSY TLP ITCQLSLGLQ SQLVPILANI LEVEQAKCWG
541 FDQFFAETS D ILQRVVVHV F SLSQAVLHHI YIHAHNTIAI FQEAVHKQTS VAPRHQEYLF
601 EGHLCVLEPS VSAQHIAHTT ASSPLTLFST AIPKGLAFRD PALDVPKFVP KVDLQADYNT
661 AKGVLGAGYQ ALRLARALD GQELMFRGLH WMEVLQATC RRTLEVARTS LLYLSSSLGT
721 ERFSSVAGTP EIQLKAAAE LRSRLRTLAE VLSRCSQNIT ETQESLSSLN RELVKS RDQV
781 HEDRSIQQIQ CCLDKMNFY KQFKKSRMRP GLGYNEEQIH KLDKVNFSHL AKRLLQVFQE
841 ECVQKYQASL VTHGKRMRV HETRNLRLV GCSVAACNTE AQGVQESLSK LLEELSHQLL
901 QDRAKGAQAS PPPIAPY P SP TRKDLLLHMQ ELCEGMKLLA SDLLDNNRII ERLNRVPAPP
961 DV

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Recombinant IKK ϵ 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
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1261 gggactgagg agtacctgca tcccagatg tatgagcggg cgggtcttcg aaagccccag

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1321 caaaaagcgt tcggggtgac tgtggatctc tggagcattg gaggacctt gtaccatgca
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