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Certificate of Analysis

IKKβ, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-485, 14-485-K, 14-485M
Parent Lot # D10JP013N

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, full-length, human IKK beta, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 71.9% by SDS-PAGE and Coomassie blue staining. MW = 87.6kDa.

Specific Activity (Parent lot# D10JP013N): 71U/mg, where one unit of IKK beta, active activity is defined as 1nmol phosphate incorporated into 100µM IKKtide (KKKKERLLDDRHDSGLDSMKDEE) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.624mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

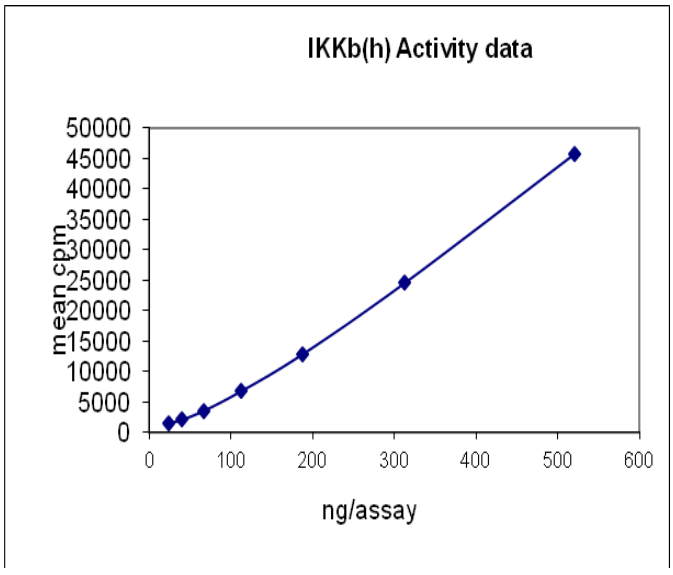
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

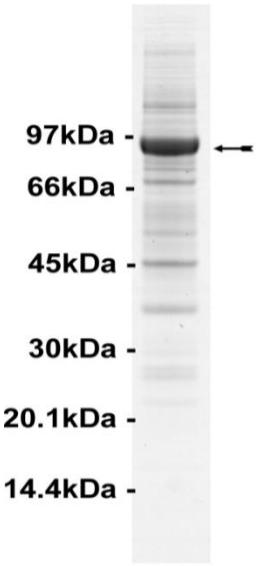
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 24–520ng of this lot of enzyme phosphorylated 100µM IKKtide 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 product identity as IKKβ 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 IKKβ, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **IKKtide (KKKKERLLDDRHDSGLDSMKDEE):** Use a final assay concentration of 100 µM. Make up a 1mM stock. Add 2.5µl of stock per assay point.
3. **IKKβ, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 24–520ng 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 **IKKtide (KKKKERLLDDRHDSGLDSMKDEE)**.
3. Add **2.5µl (24–520ng) IKKβ, active**.
4. Add 5µl of dH_2O.
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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IKKβ Sequence Information

Protein human IKKβ

Tags *N*-Terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human IKKβ

Accession number GenBank AF080158

Recombinant IKKβ amino acid sequence:

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   1 MHHHHHHMSW SPSLTTQTCG AWEMKERLGT GGFGNVIRWH NQETGEQIAI KQCRQELSPR
  61 NRERWCLEIQ IMRRLTHPNV VAARDVPEGM QNLAPNDLPL LAMEYCQGGD LRKYLNQFEN
 121 CCGLREGAIL TLLSDIASAL RYLHENRIIH RDLKPENIVL QQGEQRLIHK IIDLGYAKEL
 181 DQGSLCTSFV GTLQYLAPEL LEQQKYTVTV DYWSFGTLAF ECITGFRPFL PNWQPVQWHS
 241 KVRQKSEVDI VVSEDLNGTV KFSSSLPYPN NLNSVLAERL EKWLQLMLMW HPRQRGTDPT
 301 YGPNGCFKAL DDILNLKLVH ILNMVTGTIH TYPVTEDESL QSLKARIQQD TGIPEEDQEL
 361 LQEAGLALIP DKPATQCISD GKLNEGHTLD MDLVFLFDNS KITYETQISP RPQPESVSCI
 421 LQEPKRNLAF FQLRKVWGQV WHSIQTLKED CNRLQQGQRA AMMNLLRNNS CLSKMKNSMA
 481 SMSQQLKAKL DFFKTSIQID LEKYSEQTEF GITSDKLLLA WREMEQAVEL CGRENEVKLL
 541 VERMMALQTD IVDLQRSPMG RKQGGTLDDL EEQARELYRR LREKPRDQRT EGDSQEMVRL
 601 LLQAIQSFEK KVRVIYTQLS KTVVCKQKAL ELLPKVEEVV SLMNEDEKTV VRLQEKRQKE
 661 LWNLLKIACS KVRGPVSGSP DSMNASRLSQ PGQLMSQPST ASNSLPEPAK KSEELVAEAH
 721 NLCTLLENAI QDTVREQDQS FTALDWSWLQ TEEEEHSCLE QAS
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Recombinant IKKβ nucleotide sequence:

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   1 atgcatcatc accatcacca tatgagctgg tcaccttccc tgacaacgca gacatgtggg
  61 gcctgggaaa tgaaagagcg ccttgggaca gggggatttg gaaatgtcat ccgatggcac
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 181 aaccgagagc ggtggtgcct ggagatccag atcatgagaa ggctgaccca ccccaatgtg
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 361 tgctgtggtc tgcgggaagg tgccatcctc accttgctga gtgacattgc ctctgcgctt
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 481 cagcaaggag aacagaggtt aatacacaaa attattgacc taggatatgc caaggagctg
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 781 aagttttcaa gctctttacc ctaccccaat aatcttaaca gtgtcctggc tgagcgactg
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 901 tatgggccca atggctgctt caaggccctg gatgacatct taaacttaaa gctggttcat
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1741 ctaagggaaa aacctcgaga ccagcgaact gagggtgaca gtcaggaaat ggtacggctg
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2281 caggcctcat ga
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Reviewed and approved by site quality representative.