Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

IKK-epsilon

inhibitor of nuclear factor kappa-B kinase epsilon subunit

Recombinant Human Active Protein Kinase

HGNC Symbol: IKBKE

Synonyms: IKK-I; IKKE; IKKI

Product No.: 0320-0000-1

Lot: 007

Description: Human IKK-epsilon, full length, amino acids M_1-V_{716} (as in NCBI/Protein entry NP_054721.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: IKK-epsilon Lot 007, was confirmed as IKK-epsilon by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 110,059 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

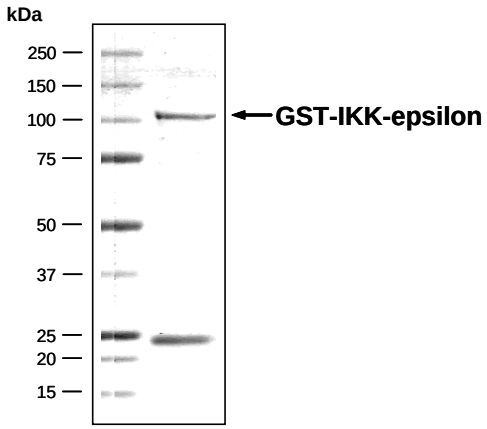
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.055 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

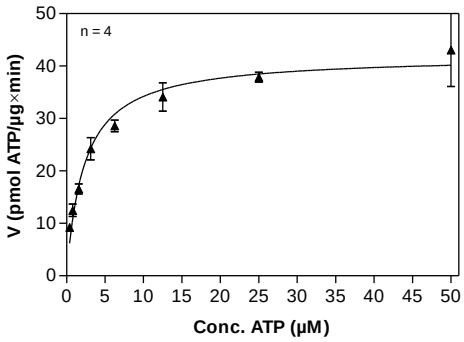
Biochemical Parameters:
Specific kinase activity (P_i transfer): 42 pmol/µg × min
ATP-K_M: 2.3 µM

IKK-epsilon Lot 007:
Coomassie stain



2.0 µg GST-IKK-epsilon

IKK-epsilon Lot 007:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: RBER-GSK3, 100 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
IKK-epsilon Lot 007 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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IKK-epsilon

Product No.: 0320-0000-1

GST-IKK-epsilon Recombinant Fusion Protein Amino Acid Sequence

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241 LVPRGSPGLD GICSRNSMQS TANYLWHTDD LLGQGATASV YKARNKKSGE LVAVKVFNTT 300
301 SYLRPREVQV REFEVLRKLN HQNIVKLFAV EETGGSRQKV LVMEYCSSGS LLSVLESPEN 360
361 AFGLPEDEFL VVLRCVVAGM NHLRENGIVH RDIKPGNIMR LVGEEGQSIY KLTDFGAARE 420
421 LDDDEKFVSV YGTEEYLHPD MYERAVLRKP QQKAFGVTVD LWSIGVTLYH AATGSLPFIP 480
481 FGGPRRNKEI MYRITTEKPA GAIAGAQRRE NGPLEWSYTL PITCQLSLGL QSQLVPILAN 540
541 ILEVEQAKCW GFDQFFAETS DILQRVVVHV FSLSQAVLHH IYIHAHNTIA IFQEAVHKQT 600
601 SVAPRHQEYL FEGHLCVLEP SVSAQHIAHT TASSPLTLFS TAIPKGLAFR DPALDVPKFV 660
661 PKVDLQADYN TAKGVLGAGY QALRLARALL DGQELMFRGL HWVMEVLQAT CRRTLEVART 720
721 SLLYLSSSLG TERFSSVAGT PEIQELKAAA ELRSRLRTLA EVLSRCSQNI TETQESLSSL 780
781 NRELVKSRDQ VHEDRSIQQI QCCLDKMNFI YKQFKKSRMR PGLGYNEEQI HKLDKVNFSH 840
841 LAKRLLQVFQ EECVQKYQAS LVTHGKRMRV VHETRNHLRL VGCSVAACNT EAQGVQESLS 900
901 KLLEELSHQL LQDRAKGAQA SPPPIAPYPS PTRKDLLLHM QELCEGMKLL ASDLLDNNRI 960
961 IERLNRVPAP PDV                                                   1020
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: IKK-epsilon

IKK-epsilon wt[1] Amino Acid Sequence

```
  1 MQSTANYLWH TDDLLGQGAT ASVYKARNKK SGELVAVKVF NTTSYLRPRE VQVREFEVLR  60
 61 KLNHQNIVKL FAVEETGGSR QKVLVMEYCS SGSLLSVLES PENAFGLPED EFLVVLRCVV 120
121 AGMNHLRENG IVHRDIKPGN IMRLVGEEGQ SIYKLTDFGA ARELDDDEKF VSVYGTEEYL 180
181 HPDMYERAVL RKPQQKAFGV TVDLWSIGVT LYHAATGSLP FIPFGGPRRN KEIMYRITTE 240
241 KPAGAIAGAQ RRENGPLEWS YTLPITCQLS LGLQSQLVPI LANILEVEQA KCWGFDQFFA 300
301 ETSDILQRVV VHVFSLSQAV LHHIYIHAHN TIAIFQEAVH KQTSVAPRHQ EYLFEGHLCV 360
361 LEPSVSAQHI AHTTASSPLT LFSTAIPKGL AFRDPALDVP KFVPKVDLQA DYNTAKGVLG 420
421 AGYQALRLAR ALLDGQELMF RGLHWVMEVL QATCRRTLEV ARTSLLYLSS SLGTERFSSV 480
481 AGTPEIQELK AAAELRSRLR TLAEVLSRCS QNITETQESL SSLNRELVKS RDQVHEDRSI 540
541 QQIQCCLDKM NFIYKQFKKS RMRPGLGYNE EQIHKLDKVN FSHLAKRLLQ VFQEECVQKY 600
601 QASLVTHGKR MRVVHETRNH LRLVGCSVAA CNTEAQGVQE SLSKLLEELS HQLLQDRAKG 660
661 AQASPPPIAP YPSPTRKDLL LHMQELCEGM KLLASDLLDN NRIIERLNRV PAPPDV      720
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blue: IKK-epsilon sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_054721.1

Recombinant Proteins

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