IKK epsilon [GST-tagged]

Kinase

Alternate Names: Inhibitor of nuclear factor kappa-B kinase subunit epsilon, I-kappa-B kinase epsilon, IKK-E, Inducible I kappa-B kinase, IKBKE, IKKI, KIAA0151

Cat. No. **66-0038-050**
Lot. No. **30317**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Inhibitor of IκB kinases (IKK) are key regulators of NF-κB signalling. Three IKK isoforms-α, β, and ε-have been linked to oncogenesis (Hsu *et al.*, 2012). IKK epsilon (IKKε) is a key regulator of innate immunity and a breast cancer oncogene, amplified in ~30% of breast cancers, that promotes malignant transformation through NF-κB activation (Zhou *et al.*, 2013). Cloning of the IKK epsilon gene was first described by Shimada *et al.* (1999). IKK epsilon can be modified and regulated by K63-linked polyubiquitylation at lysine 30 and lysine 401. Tumour necrosis factor alpha (TNFα) and interleukin-1β (IL-1β) stimulation can induce IKK epsilon K63-linked polyubiquitylation, and this modification is essential for IKK epsilon kinase activity, IKK epsilon-mediated NF-κB activation, and IKK epsilon-induced malignant transformation. Disruption of K63-linked ubiquitylation of IKK epsilon does not affect its overall struc-

Continued on page 2

Physical Characteristics

Species: human

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 0.34 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~107.3 kDa

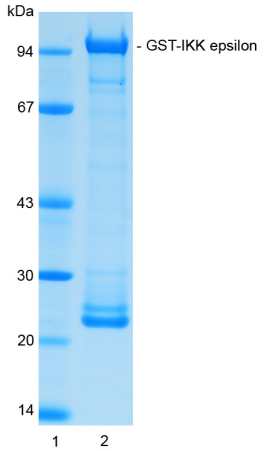
Purity: >60% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg GST-IKK epsilon



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-IKK epsilon was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-IKK epsilon was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of MBP substrate (1 mg/ml) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate.The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-IKK epsilon specific activity:
561.7 Units/mg (190.9 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: Myelin Basic Protein (MBP)

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

ture but impairs the recruitment of canonical NF-κB proteins. The ubiquitin E3 ligase complex involved in binding to and ubiquitylating IKK epsilon is cIAP1/cIAP2/TRAF2 (Zhou *et al.*, 2013).

References:

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Hsu S, Kim M, Hernandez L, Grajales V, Noonan A, Anver M, *et al.* (2012) IKK-epsilon coordinates invasion and metastasis of ovarian cancer. *Cancer Res* **72**, 5494-5504.

Shimada T, Kawai T, Takeda K, Matsumoto M, Inoue J, Tatsumi Y, *et al.* (1999) IKK-i, a novel lipopolysaccharide-inducible kinase that is related to IkappaB kinases. Int Immunol **11**, 1357-1362.

Zhou AY, Shen RR, Kim E, Lock YJ, Xu M, Chen ZJ, *et al.* (2013) IKKepsilon-mediated tumorigenesis requires K63-linked polyubiquitination by a cIAP1/cIAP2/TRAF2 E3 ubiquitin ligase complex. *Cell Rep* **3**, 724-733.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCP
KERAEISMLEGAVLDIRYGVSRIAYSKD
FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG
WQATFGGGDHPPKSDLEVLFQGPLGS***M***QSTA**
NYLWHTDDLLGQGATASVYKARNKKSGEL
VAVKVFNTTSYLRPREVQVREFEVLRKLN
HQNIVKLFAVEETGGSRQKVLVMEYCSSG
SLLSVLESPENAFGLPEDEFLVVLRCVVAG
MNHLRENGIVHRDIKPGNIMRLVGEEGQSI
YKLTDFGAARELDDDEKFVSVYGTEEYLH
PDMYERAVLRKPQQKAFGVTVDLWSIGVT
LYHAATGSLPFIPFGGPRRNKEIMYRIT
TEKPAGAIAGAQRRENGPLEWSYTLPITC
QLSLGLQSQLVPILANILEVEQAKCWGFDQF
FAETSDILQRVVVHVFSLSQAVLHHIYIHAHN
TIAIFQEAVHKQTSVAPRHQEYLFEGHLCV
LEPSVSAQHIAHTTASSPLTLFSTAIPKGLA
FRDPALDVPKFVPKVDLQADYNTAKGVL
GAGYQALRLARALLDGQELMFRGLHWVMEV
LQATCRRTLEVARTSLLYLSSSLGTERFSS
VAGTPEIQELKAAAELRSRLRTLAEVLSRC
SQNITETQESLSSLNRELVKSRDQVHEDR
SIQQIQCCLDKMNFIYKQFKKSRMRPGLGYNE
EQIHKLDKVNFSHLAKRLLQVFQEECVQKY
QASLVTHGKRMRVVHETRNHLRLVGCS
VAACNTEAQGVQESLSKLLEELSHQLLQDRAK
GAQASPPPIAPYPSPTRKDLLLHMQELCEGM
KLLASDLLDNNRIIERLNRVPAPPDV

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
IKK epsilon (regular text): Start ***bold italics*** (amino acid residues 1-716).
Accession number: NP_054721

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