IKK epsilon pSer172 (human; residues 168–177), pAb

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.	**68-0052-100**	Quantity:	100 µg
Lot. No.	**30292**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

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 (IKKs) 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-

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human IKK epsilon (residues 168 – 177) [CEKFV(pS)VYGTE]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects IKK epsilon at ~81 kDa

Reactivity: human

Species cross reactivity: mouse

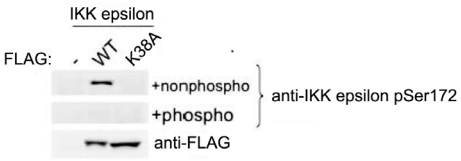
Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

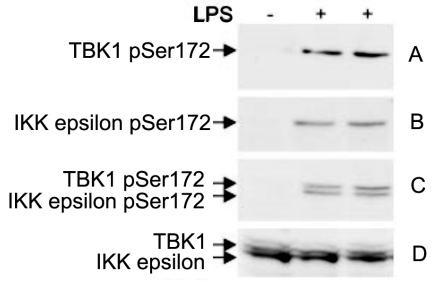
Western Immunoblotting:
use 1 µg/ml

Immunoprecipitation:
use 10 µg/mg

Add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1008-001 provided) to your immunoblotting or immunoprecipitation incubation per 1 µg of polyclonal antibody in order to deplete any non-phospho specific polyclonal antibodies present.



Western Blotting Analysis:
HEK293 cells were transfected with constructs for FLAG-tagged wildtype (WT) and kinase dead (K38A) IKK epsilon. The transfected IKK epsilon was immunoprecipitated from cell extracts using commercially available anti-FLAG M2 agarose and immunoblotted with anti-FLAG and anti-IKK epsilon pSer172 antibodies (Cat# 68-0052-100) in the presence of either non-phosphorylated or phosphorylated peptide.



Immunoprecipitation Assay:
RAW264.7 cells were unstimulated (-) or stimulated (+) with 100 ng/ml LPS for 30 mins. 1 mg of cellular extract was subjected to immunoprecipitation using 10 µg anti-IKK epsilon pSer172 (Cat# 68-0052-100) in the presence of 100 µg of non-phosphorylated peptide. IKK epsilon - and TBK1 - were subsequently detected by Western Blot using anti-TBK1 pSer172 (Panel A; Cat# 68-0054-100), anti-IKK pSer172 (Panel B; Cat# 68-0052-100), and commercially available anti-TBK1 (Panel C) and anti-IKK epsilon (Panel D) antibodies.

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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

IKK epsilon pSer172 (human; residues 168–177), pAb

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.	**68-0052-100**	Quantity:	100 µg
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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

mediated NF-κB activation, and IKK epsilon-induced malignant transformation. Disruption of K63-linked ubiquitylation of IKK epsilon does not affect its overall structure 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).

Antibody Production:

Anti-IKK epsilon pSer172 (human) polyclonal antibody was raised in sheep against IKK epsilon pSer172 (residues 168-177 of human IKK epsilon; Ser172 phosphorylated). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-IKK epsilon pSer172 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-IKK epsilon pSer172 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

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General References:

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.

Application Reference:

Clark K, Plater L, Peggie M and Cohen P (2009) Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. *J Biol Chem* **284**, 14136-14146.

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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