

IKK epsilon (human; residues 701–716), 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. 68-0050-100
Lot. No. 30290

Quantity: 100 µg
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

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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-

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: IKK epsilon (residues 701-716) [NRRIERLNRPAPPDV]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects IKK epsilon at ~81 kDa

Reactivity: human

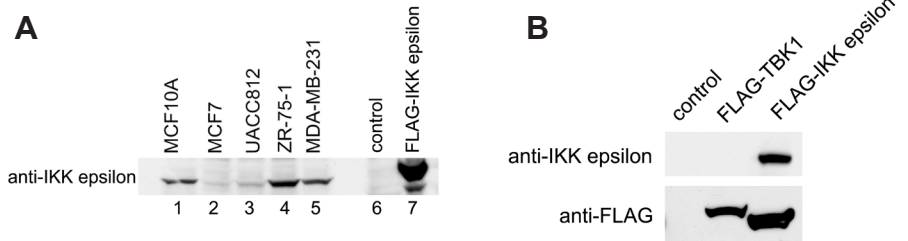
Species cross reactivity: mouse

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

Research Applications and Quality Assurance

Western Immunoblotting: use 1 µg/ml

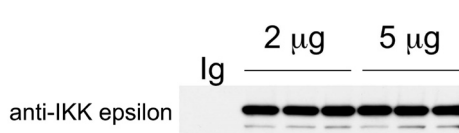
Immunoprecipitation: use 2 µg/mg of cell extract



Western Blotting Analysis:

A) IKK epsilon was detected by Western Blotting from 25 µg of cell lysates from various breast cancer cell lines (Lanes 1-5) or HEK293 cells transfected with either empty vector (Lane 6; negative control) or FLAG-tagged IKK epsilon (Lane 7). The membrane was immunoblotted with 1 µg/ml anti-IKK epsilon antibody (Cat# 68-0050-100).

B) 25 µg HEK293 cells were transfected with empty vector (control), FLAG-tagged TBK1 or FLAG-tagged IKK epsilon. The membrane was immunoblotted with 1 µg/ml anti-IKK epsilon antibody (Cat# 68-0050-100) or a commercially available anti-FLAG antibody.



Immunoprecipitation Assay:

Immunoprecipitation was performed from RAW264.7 macrophages total cell extract (1 mg) using 2 or 5 µg of anti-IKK epsilon antibody (Cat# 68-0050-100) or pre-immune serum (Ig). IKK epsilon was subsequently detected by Western Blot using a commercially available anti-IKK epsilon antibody.



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Lot-specific COA version tracker: v1.0.0



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Background

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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 (human) polyclonal antibody was raised in sheep against IKK epsilon (residues 701-716 of human IKK epsilon). 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 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-IKK epsilon (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

Application References:

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 IκappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. *J Biol Chem* **284**, 14136-14146.

Clark K, Takeuchi O, Akira S and Cohen P (2011) The TRAF-associated protein TANK facilitates cross-talk within the IκappaB kinase family during Toll-like receptor signaling. *Proc Natl Acad Sci USA* **108**, 17093-17098.

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-ι, a novel lipopolysaccharide-inducible kinase that is related to IκappaB 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.



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