

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and **Ubiguitylation 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. IKK is a serine protein kinase and the IKK complex contains α and β subunits termed IKK α and IKK β . The catalytic activities of both IKK α and IKKB make essential contributions to IkB phosphorylation and NF-kB activation (Zandi et al., 1997). Cloning of human IKKB was described in 1997 by a number of groups (Mercurio et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). IKKß shares 50% identity with IKK α and like it contains a kinase domain, a leucine zipper, and a helix-loop helix (Zandi et al., 1997). IKKβ can phosphorylate and modulate the functions of several cytosolic proteins, including β-catenin, SRC-3, FOXO3a, CYLD and DOK1 (Lee et al., 2007). IKKB is phosphorylated for activation by several factors, such as pro-inflammatory cytokines and

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Lot-specific COA version tracker: v1.0.0

IKK beta (human; full length), pAb

Alternate Names: Inhibitor of nuclear factor kappa-B kinase subunit beta, I-kappa-B-kinase beta, IKK-B, IkBKB, I-kappa-B kinase 2, IKK2

Cat. No.	68-0049-100
Lot. No.	30288

FOR RESEARCH USE ONLY

Storage:

NOT FOR USE IN HUMANS

100 µg -20°C

CERTIFICATE OF ANALYSIS

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Quantity:

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human IKK beta (residues 1-736) [6His-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects IKK beta at ~87 kDa

Reactivity: human

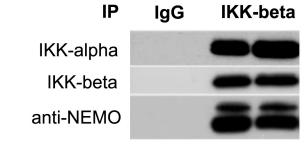
Species cross reactivity: mouse

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

Research Applications and Quality Assurance

Western Immunoblotting: not tested

Immunoprecipitation: use 10 µg/mg of cell extract



Immunoprecipitation Assay:

Immunoprecipitation was performed from RAW264.7 macrophage total cell extract (1 mg) using 10 µg of anti-IKK beta antibody (Cat# 68-0049-100 or pre-immune serum (IgG). IKK beta (as well as IKK alpha and NEMO) were subsequently detected by Western Blot using commercially available antibodies.

N.B: As IKK beta is a component of the canonical IKK complex, other components of the complex (the catalytic subunits IKK alpha and NEMO) are also immunoprecipitated with the anti-IKK beta antibody.



IKK beta (human; full length), pAb

Alternate Names: Inhibitor of nuclear factor kappa-B kinase subunit beta, I-kappa-B-kinase beta, IKK-B, IkBKB, I-kappa-B kinase 2, IKK2

Cat. No. Lot. No.

68-0049-100 30288 Quantity: Storage: 100 µg -20°С

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CERTIFICATE OF ANALYSIS

IKK beta suppression of TSC1 links inflammation and tumor angio-

Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, et

al. (1997) IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. Science 278, 860-866.

Wada K, Niida M, Tanaka M and Kamitani T (2009) Ro52-mediated monoubiquitination of IKK{beta} down-regulates NF-{kappa}B sig-

Woronicz JD, Gao X, Cao Z, Rothe M and Goeddel DV (1997) Ikap-

paB kinase-beta: NF-kappaB activation and complex formation with lkappaB kinase-alpha and NIK. Science 278, 866-869.

Zandi E, Rothwarf DM, Delhase M, Hayakawa M and Karin M (1997)

The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and

Zhang J, Clark K, Lawrence T, Peggie MW and Cohen P (2014) An unexpected twist to the activation of IKKbeta: TAK1 primes IKKbeta

for activation by autophosphorylation. Biochem J 461, 531-537.

genesis via the mTOR pathway. Cell 130, 440-455.

nalling. J Biochem 146, 821-832

NF-kappaB activation. Cell 91, 243-252.

Application Reference:

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Background

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the Tax oncoprotein of HTLV-1. In cells infected by HTLV-1, IKKβ is persistently phosphorylated and conjugated with monoubiquitin due to Tax expression. Although this Tax-induced monoubiquitylation appears to be an important regulation system for IKKB, how the monoubiguitylation occurs is unknown and its role in NF-kB signalling is still unclear (Wada et al., 2009). TNFα mediates tumour angiogenesis through dysregulated mTOR signalling caused by suppression of TSC1 by IKKB, revealing a novel mechanism of inflammation-mediated tumour angiogenesis. Further investigations of the IKKB/TSC1/mTOR signalling pathway may identify new molecules involved in regulating TNFα-induced pathological disorders. Preventing the phosphorylation of TSC1 may have important clinical implications for the treatment or prevention of cancer (Lee et al., 2007).

Antibody Production:

Anti-IKK beta (human) polyclonal antibody was raised in sheep against IKK beta (residues 1-736 of human IKK beta). 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 beta pAbs from the sheep serum using a 6His-tagged antigen-agarose column. Anti-IKK beta (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, et al. (2007)



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