University of Dundee

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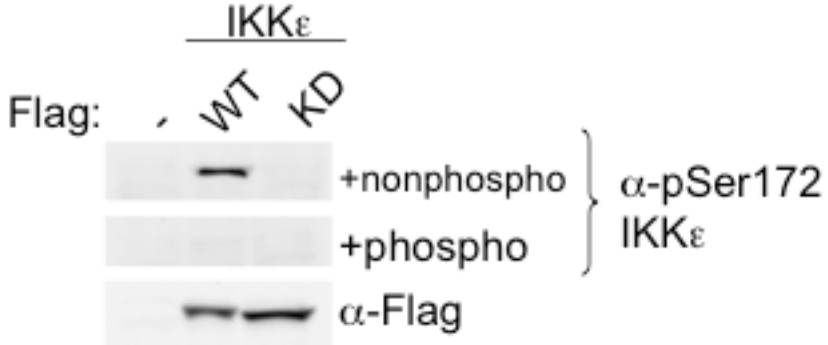
Antibody Datasheet

Product description:-	Anti-IKK epsilon phospho Ser 172
Antigen:-	CEKFVS*VYGTE [residues 168 – 177 of human, plus N-terminal cysteine residue for coupling, where * is phospho Serine]
Sheep Number:-	S051C
Bleed Number:-	Second Bleed
Concentration:-	0.24 mg/ml
Formulation:-	Phosphate Buffered Saline
Storage temperature:-	–20 °C
Purification Method:-	Affinity purified against phosphorylated specific peptide
Working Concentration:-	1 μg/ml for immuno-blotting in the presence of 10 ug/ml of the non-phosphorylated peptide 10 μg antibody + 100 μg non-phosphorylated peptide per mg of cellular extract for immunoprecipitation of the phosphorylated form of IKK epsilon and TBK1
Publication Reference:-	Unpublished – Therefore please treat this information as highly confidential

(A) Immunoblotting with anti-IKK epsilon phospho Ser 172

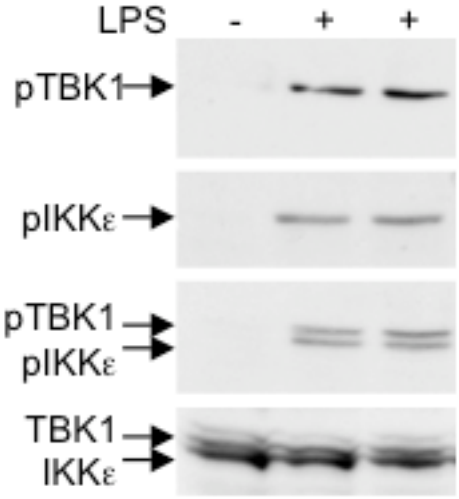


HEK 293 cells were transfected with constructs for Flag tagged wildtype and kinase dead (K38A) IKK epsilon. The transfected IKK epsilon was immunoprecipitated from cell extracts using anti-Flag M2 agarose (Sigma). The resulting immunoprecipitate was subjected to SDS-Page followed by immunoblotting with anti-FLAG (Sigma) and anti-

IKK phospho Ser 172 antibodies (in the presence of non-phosphorylated or phosphorylated peptide).

Binding of IKK epsilon phospho Ser 172 antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).

(B) Immunoprecipiation of phosphorylated IKK epsilon and TBK1 using anti-IKK epsilon phospho Ser 172



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 ug anti-IKK epsilon phospho Ser 172 in the presence of 100 ug of non-phosphorylated peptide. The resulting immunoprecipitate was subjected to SDS-Page followed by immunoblotting with anti-TBK1 phospho Ser 172, anti-IKK phospho Ser 172, anti-TBK1 (CST) and anti-IKK epsilon (Sigma).

Binding of TBK1 and IKK epsilon phospho specific antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).