University of Dundee

Contact:- Dr C. James Hastie

Antibody Datasheet

Product description:- Anti-IKK epsilon (701 – 716)

Antigen:- NRIIERLNRVPAPPDV
[residues 701 – 716 of human]

Sheep Number:- S255C

Bleed Number:- Second Bleed

Concentration:- 0.37 mg/ml

Formulation:- Phosphate Buffered Saline

Storage temperature:- –20 °C

Purification Method:- Affinity purified against peptide NRIIERLNRVPAPPDV

Working Concentration:- 1 μg/ml for immuno blotting

2 μg per mg of extract for immunoprecipitation

Publication Reference:- Not published with this antibody

Notes:
This antibody is specific for IKK epsilon and does not recognise TBK1. Although it was raised against the human sequence of IKK epsilon, it recognises both the human and mouse sequences of IKK epsilon.

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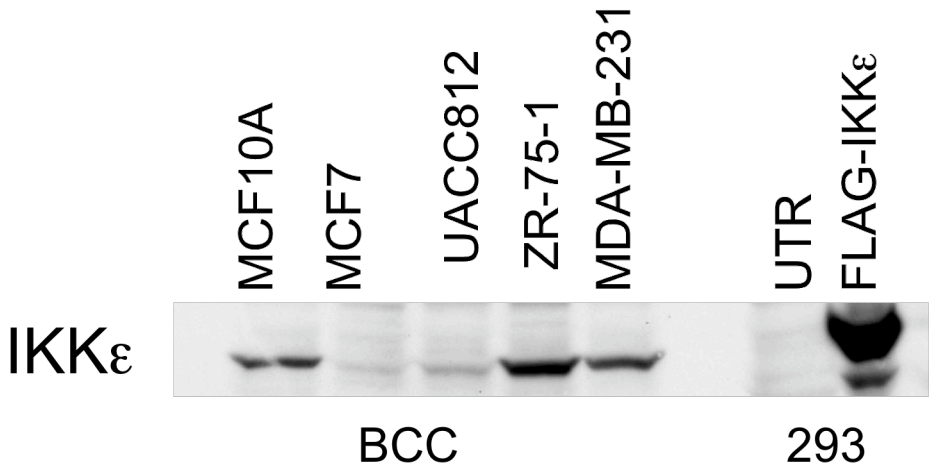
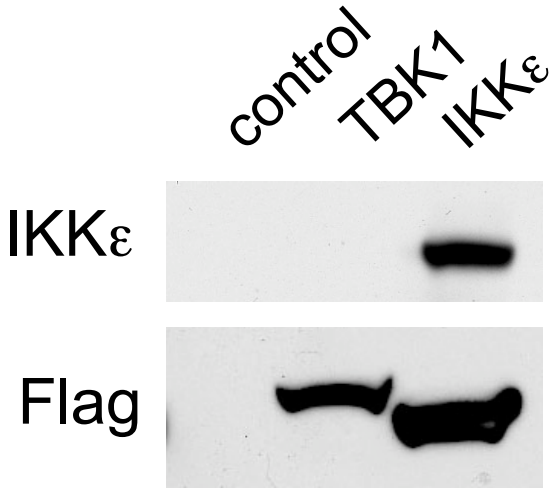
Figure One:



25 ug of cell lysates from various breast cancer cell lines and HEK293 cells transfected with either empty vector (UTR) or FLAG tagged IKK epsilon were separated on an 8 % SDS-Page gel and transferred to PDVF membrane. The membrane was immunoblotted with anti-IKK epsilon (701 – 716) at 1 ug/ml. Binding of the primary antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).

Figure Two:



25 ug of HEK293 cells transfected with either empty vector (control), FLAG tagged TBK1 or FLAG tagged IKK epsilon were separated on an 8 % SDS-Page gel and transferred to PDVF membrane. The membrane was immunoblotted with anti-IKK epsilon (701 – 716) at 1 ug/ml (upper panel) or anti-FLAG M2 [Sigma] (lower panel). Binding of the primary antibody was detected using rabbit peroxidase conjugated anti-sheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).

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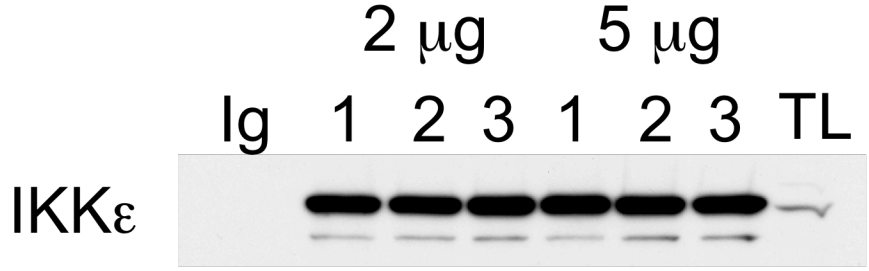
Figure Three:



1 mg of protein extract from RAW264.7 macrophages was incubated with pre-immune IgG (Ig) or either the first, second or third bleeds of affinity purified anti-IKK epsilon (701 – 716) at 2 or 5 ug. The samples were incubated for 1 hr at 4 deg C on an end over end roller. 10 ul of Protein G Sepharose was added and incubated for a further 15 mins at 4 deg C. The beads were collected by centrifugation and washed three times in lysis buffer. The immunoprecipitates were separated on an 8 % SDS-Page gel, along with 20 ug of total protein extract (TL), and transferred to PDVF membrane. The membrane was immunoblotted with anti-IKK epsilon (Sigma) followed by HRP-conjugated anti-mouse antibodies with detection by enhanced chemiluminescence (ECL, Amersham).