Antibody Quality Control of non-phospho antibodies:

Name of Antibody: anti-IKKe, residues 702-717 of mouse IKKe (NRLIERLHRVPSAPDV)

Sheep Number: S277C

Bleed Number: 1+2+3

Date Purified: 15/10/07

Immuno Blotting:

Does it recognise endogenous protein without immunoprecipitation:
NOT TESTED but it does detect overexpressed IKKe very well by Western blotting.

Concentration used: 1 μg/ml

Immunoprecipitation:

Does it immunoprecipitate endogenous protein:
Yes, I can completely deplete IKKε from the lysates of RAW264.7 mouse macrophages.

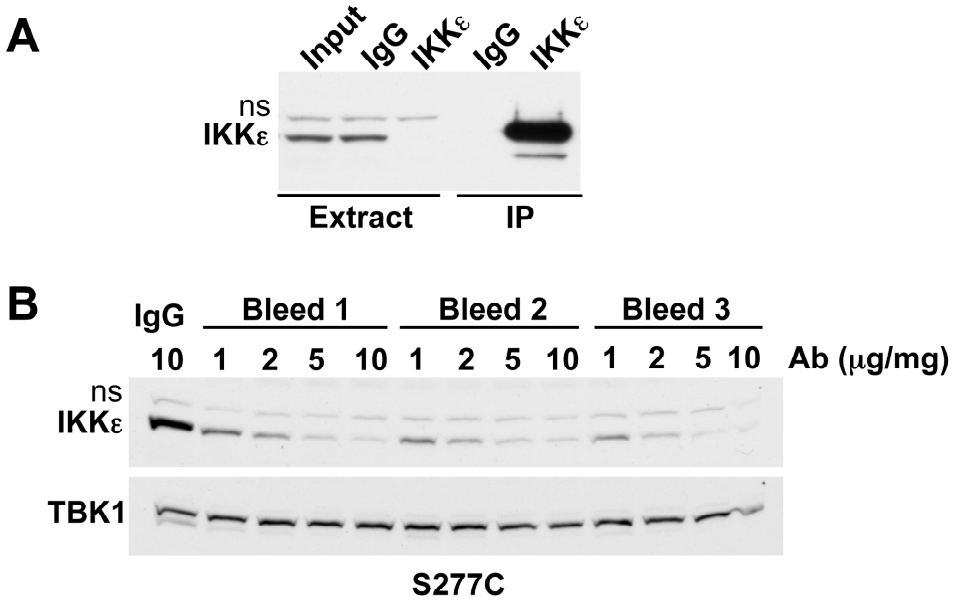
Amount used: lowest amount tested was 1 μg of Ab/ mg of protein extract

NOTES: Antibody is specific for IKKε and does not immunodeplete TBK1. Although raised against the mouse IKKε peptide sequence, antibody recognizes both mouse and human IKKε.

References:

Clark et al. (2009) Use of the Pharmacological Inhibitor BX795 to Study the Regulation and Physiological Roles of TBK1 and IκB Kinase ε: A DISTINCT UPSTREAM KINASE MEDIATES SER-172 PHOSPHORYLATION AND ACTIVATION. J Biol Chem 284, pp. 14136-46

Clark et al. (2011) The TRAF-associated protein TANK facilitates cross-talk within the IκB kinase family during Toll-like receptor signalling. Proc. Natl. Acad. Sci. U. S. A. (in press)



(A) Lysates (1 mg) of RAW264.7 macrophages were incubated with antibodies from non-immunised sheep (IgG) or antibodies against IKK epsilon (S277C, 2nd bleed). After one hour incubation, protein G-Sepharose was added for a further 15 min. The supernatant was removed and depletion of IKK epsilon was analyzed by immmoblotting (extract). The immunoprecipitates were washed and immunoblotted (IP). NS = non-specific band

(B) Lysates (1 mg) of RAW264.7 macrophages were incubated with antibodies from non-immunised sheep (IgG) or antibodies against IKK epsilon (S277C, 2nd bleed). After one hour incubation, protein G-Sepharose was added for a further 15 min. After brief centrifugation to pellet the protein G-Sepharose, the supernatant was removed and depletion of IKK eplison was analysed by immunoblotting.
[Note that the related kinase TBK1 was not immunodepleted by the amtibody. Immunoblotting was performed using TBK1 and IKK epsilon antibodies purchased from Cell Signalling Technology]