Antibody Quality Control of non-phospho antibodies:

Name of Antibody: IKK beta (human)

Sheep Number: S189C

Bleed Number: 1-3

Immuno Blotting: NOT TESTED

Immunoprecipitation: YES

Does it immunoprecipitate endogenous protein: YES, RAW264.7 (mouse cell line) and primary mouse macrophages. The antibody successfully immunoprecipitated the entire canonical IKK complex.

Amount used: 3-10 μ g of bleed 1-3 with 1 mg of cell extract. Immunoblotted with a commercial antibody from Millipore. NOTE that bleed 1 works best and that the antibody does not detect IKK α , IKK ϵ or TBK1.

Immuno blotting after Immunoprecipitation: NOT TESTED

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

IP	IgG	ΙΚΚβ
IKKα		
ΙΚΚβ		
NEMO		3=

Lysates of RAW264.7 macrophages (1 mg cell extract) were incubated with antibodies (10 mg) from non-immunised sheep (IgG) or antibodies (10 mg) against IKKb. The immunoprecipitates were washed, separated by SDS-PAGE and immunoblotted with the antibodies indicated.

IKKb is part of the canonical IKK complex which consists of NEMO, IKKa and IKKb. Note that the antibody against IKKb immunoprecipitates the entire canonical IKK complex.