TBK1 pSer172 (human; residues 168-177), pAb

Alternate Names: TANK-BINDING Kinase 1; NF-Kappa-B-Activating Kinase; NAK

Cat. No.	**68-0054-100**	Quantity:	100 µg
Lot. No.	**30294**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation 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 post-translational 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. Cloning of human TANK-binding kinase 1 (TBK1) was first described by Pomerantz and Baltimore (1999). TBK1 is an IKK-related kinase, which plays several important roles in the innate immune system. In the MyD88-dependent signalling pathway its activation requires the E3 ubiquitin ligase TRAF6 and the polyubiquitin-binding protein NEMO (Clark *et al.*, 2011a; Clark *et al.*, 2011b). TBK1 interacts with and phosphorylates the NEMO-related protein optineurin (Gleason *et al.*, 2011). TBK1 also plays an essential role in production of type1 interferons that are produced in response to viral double-stranded RNA. This is triggered by the TBK1-catalysed activation of the transcription factor IRF3 (interferon regulatory factor 3) and the E3 ubiquitin ligase Pellino

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human TBK1 (residues 168 – 177) [CEQFV(pS)LYGTE]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects TBK1 at ~111 kDa

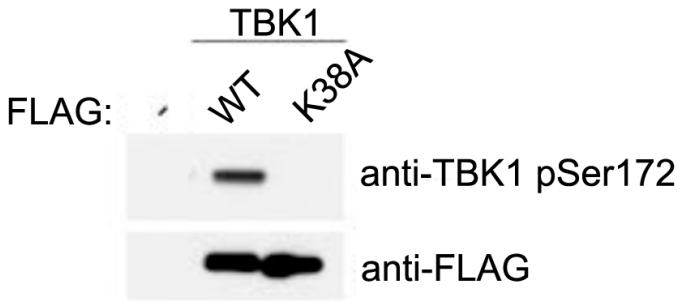
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
use 1 µg/ml; add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1009-001 provided) to your immunoblotting incubation per 1 µg of polyclonal antibody in order to deplete any non-phospho specific polyclonal antibodies present.

Immunoprecipitation:
not tested



Western Blotting Analysis:
HEK293 cells were transfected with constructs coding for FLAG-tagged wildtype (WT) or kinase dead (K38A) TBK1. The transfected TBK1 was immunoprecipitated from cell extracts using anti-FLAG M2 agarose and immunoblotted with either commercially available anti-FLAG antibody or anti-TBK1 pSer172 antibody (Cat# 68-0054-100) in the presence of the non-phosphorylated peptide.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

1 (Perry *et al.*, 2004; Smith *et al.*, 2011). TBK1 can be used to activate E3 ubiquitin ligases of the Pellino family *in vitro* (Smith *et al.*, 2011). TBK1 itself contains a ubiquitin-like domain situated next to the kinase catalytic domain which appears to be important for the activation of and/or substrate recognition by the protein kinase (Ikeda *et al.*, 2007).

Antibody Production:

Anti-TBK1 pSer172 (human) polyclonal antibody was raised in sheep against TBK1 pSer172 (residues 168-177 of human TBK1; Ser172 phosphorylated). 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-TBK1 pSer172 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-TBK1 pSer172 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Clark K, Peggie M, Plater L, Sorcek RJ, Young ER, Madwed JB, *et al.* (2011) Novel cross-talk within the IKK family controls innate immunity. *Biochem J* **434**, 93-104.

Clark K, Takeuchi O, Akira S and Cohen P (2011) The TRAF-associated protein TANK facilitates cross-talk within the IkappaB kinase family during Toll-like receptor signaling. *Proc Natl Acad Sci USA* **108**, 17093-17098.

Gleason CE, Ordureau A, Gourlay R, Arthur JS and Cohen P (2011) Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon beta. *J Biol Chem* **286**, 35663-35674.

Ikeda F, Hecker CM, Rozenknop A, Nordmeier RD, Rogov V, Hofmann K, *et al.* (2007) Involvement of the ubiquitin-like domain of TBK1/IKK-i kinases in regulation of IFN-inducible genes. *EMBO J* **26**, 3451-3462.

Perry AK, Chow EK, Goodnough JB, Yeh WC and Cheng G (2004) Differential requirement for TANK-binding kinase-1 in type I interferon responses to toll-like receptor activation and viral infection. *J Exp Med* **199**, 1651-1658.

Pomerantz JL and Baltimore D (1999) NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. *EMBO J* **18**, 6694-6704.

Smith H, Liu XY, Dai L, Goh ET, Chan AT, Xi J, *et al.* (2011) The role of TBK1 and IKKepsilon in the expression and activation of Pellino 1. *Biochem J* **434**, 537-548.

Application References:

Clark K, Plater L, Peggie M and Cohen P (2009) Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. *J Biol Chem* **284**, 14136-14146.

Clark K, Takeuchi O, Akira S and Cohen P (2011) The TRAF-associated protein TANK facilitates cross-talk within the IkappaB kinase family during Toll-like receptor signaling. *Proc Natl Acad Sci USA* **108** 17093-17098.

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