TBK1 [GST-tagged]

Kinase

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

Cat. No.	**66-0016-050**	Quantity:	50 µg
Lot. No.	**2123**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background by Sir Philip Cohen

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational 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 signaling 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 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

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Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 270 mM sucrose, 0.03% Brij, 0.1% β-Mercapto-ethanol, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~110.5 kDa

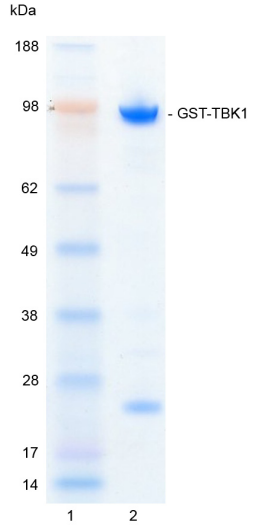
Purity: >85% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-TBK1



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-TBK1 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-TBK1 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of the EP3701 peptide substrate (300 µM) and [γ-^{32}P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-TBK1 specific activity:
221.0 Units/mg (221.0 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: EP3701 (KKKKERLLDDRHDSGLDSMKDEE)

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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 by Sir Philip Cohen

Continued from page 1

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).

References:

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

Clark K, Takeuchi O, Akira S, Cohen P (2011b) The TRAF-associated protein TANK facilitates cross-talk within the IkappaB kinase family during Toll-like receptor signaling. *Proc Natl Acad Sci U S A* **108**, 17093-8.

Gleason CE, Ordureau A, Gourlay R, Arthur JS, 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**, 35663-74.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Ikeda F, Hecker CM, Rozenknop A, Nordmeier RD, Rogov V, Hofmann K, Akira S, Dotsch V, Dikic I (2007) Involvement of the ubiquitin-like domain of TBK1/IKK-i kinases in regulation of IFN-inducible genes. *EMBO J* **26**, 3451-62.

Perry AK, Chow EK, Goodnough JB, Yeh WC, 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**, 1651-8.

Pomerantz JL, Baltimore D (1999) NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. *EMBO J* **18**, 6694-704.

Smith H, Liu XY, et al. (2011) The role of TBK1 and IKKepsilon in the expression and activation of Pellino 1. *Biochem J* **434**, 537-48.

Background kindly written by:

Sir Philip Cohen FRS, FRSE
University of Dundee

Director of the Medical Research Council Protein Phosphorylation Unit (1990-2012)

Director of the Scottish Institute for Cell Signalling incorporating the Protein Ubiquitylation Unit (2008-2012)

Co-Director of the Division of Signal Transduction Therapy (1998-2012)

Deputy Director of the Division of Signal Transduction Therapy (from July 2012)

Professor Cohen's research group is studying the interplay between protein phosphorylation and protein ubiquitylation in the regulation of innate immunity.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGS***M***QSTSN
HLWLLSDILGQGATANVFRGRHKKTGDL
FAIKVFNNISFLRPVDVQMREFEVLKKLNH
KNIVKLFAIEEETTTRHKVLIMEFCPCGSLYT
VLEEPSNAYGLPESEFLIVLRDVVGGMNHL
RENGIVHRDIKPGNIMRVIGEDGQSVYKLTDF
GAARELEDDEQFVSLYGTEEYLHPDMYERAV
LRKDHQKKYGATVDLWSIGVTFYHAATGSLP
FRPFEGPRRNKEVMYKIITGKPSGAISGVQ
KAENGPIDWSGDMPVSCSLSRGLQVLLTPV
LANILEADQEKCWGFDQFFAETSDILHRMVIH
VFSLQQMTAHKIYIHSYNTATIFHELVYKQT
KIISSNQELIYEGRRLVLEPGRLAQHFPKT
TEENPIFVVSREPLNTIGLIYEKISLPKVH
PRYDLDGDASMAKAITGVVCYACRIASTLL
LYQELMRKGIRWLIELIKDDYNETVHKKTEV
VITLDFCIRNIEKTVKVYEKLMKINLEAAEL
GEISDIHTKLLRLSSSQGTIETSLQDIDSRL
SPGGSLADAWAHQEGTHPKDRNVEKLQVLLNC
MTEIYYQFKKDKAERRLAYNEEQIHKFDKQK
LYYHATKAMTHFTDECVKKYEAFLNKSEE
WIRKMLHLRKQLLSLTNQCFDIEEEVSKY
QEYTNELQETLPQKMFTASSGIKHTMTPI
YPSSNTLVEMTLGMKKLKEEMEGVVKELAEN
NHILERFGSLTMDGGLRNVDCL

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
TBK1 (regular text): Start ***bold italics*** (amino acid residues 1-729)
Accession number: NP_037386

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