TAK1-TAB1 [6His-tagged]

Kinase

Alternate Names: Nuclear Hormone Receptor TR4; TR4: MAP3K7IP1;TAK1-Binding Protein 1

Cat. No.	**66-0007-050**	Quantity:	50 µg
Lot. No.	**2132**	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 TAK1 (TGFβ-activated protein kinase 1) was first described by Kondo *et al.* (1998). *In vivo*, TAK1 activation requires its association with TAK1 binding protein 1 (TAB1), which triggers TAK1 autophosphorylation at Thr184 and Thr187 (Sakurai *et al.*, 2000; Shibuya *et al.*, 1996). TAK1 plays a central role in the innate immune system by activating the canonical IKK complex and hence the transcription factor NFκB, as well as several MAP kinase cascades. Its activation in the MyD88 signaling pathway of the innate immune system depends on TRAF6. This E3 ubiquitin ligase generates Lys63-linked polyubiquitin chains that interact with the TAB2 and TAB3 regulatory components of the TAK1 complex. This induces a conformational change that allows TAK1 to activate itself (Wang *et al.*, 2001; Xia *et al.*, 2009).

Continued on page 2

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-Mercaptoethanol, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~44.5 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

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

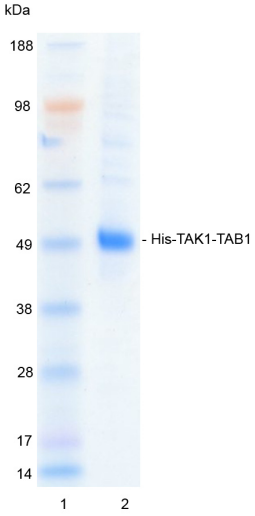
Protein Sequence:

MSYYHHHHHHDYDIPTTENLYFQGAMGS***M***
STASAASSSSSSSAGEMIEAPSQVLNFEE
IDYKEIEVEEVVGRGAFGVVCKAKWRAKDVAI
KQIESESERKAFIVELRQLSRVNHPNIVKLYGA
CLNPVCLVMEYAEGGSLYNVLHGAEPLPYYTAA
HAMSWCLQCSQGVAYLHSMQPKALIHRDLKP
PNLLLVAGGTVLKICDFGTACDIQTHMTNNKG
SAAWMAPEVFEGSNYSEKCDVFSWGIILWEVIT
RRKPFDEIGGPAFRIMWAVHNGTRPPLIKNLPK
PIESLMTRCWSKDPSQRPSMEEIVKIMTHLMRYF
PGADEPLQYPCQ**Q**SPTLTLQSTNTHTQSSSSSS
DGGLFRSRPAHSLPPGEDGRVEPYVDFAEFYRL
WSVDHGEQSVVTAP

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYF▼QG)
TAK1-TAB1 (regular text): TAK1 Start ***bold italics*** (amino acid residues 1-303); TAB1 Start ***bold italics*** (amino acid residues 437-504)
Accession numbers: TAK1: NP_003179.1; TAB1: NP_006107.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-TAK1-TAB1



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-TAK1-TAB1 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-TAK1-TAB1 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of EP3151 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.

His-TAK1-TAB1 specific activity:
92.5 Units/mg (92.5 Units/ml)

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

Substrate: EP3151 (RLGRDKYKTLRQIRQ)

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Lot-specific COA version tracker: v1.0.0

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Background by Sir Philip Cohen

Continued from page 1

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Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, Ueno N, Irie K, Nishida E, Matsumoto K (1996) TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. *Science* **272**, 1179-82.

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Background kindly written by:

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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.

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