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Catalog #	Aliquot Size
M15-13G-05	5 µg
M15-13G-10	10 µg

TAK1-TAB1, Active

Human fusion recombinant protein expressed in Sf9 cells

Catalog # M15-13G
Lot # T1064-3

Product Description

Recombinant human proteins TAK1 (1-303) and TAB1 (437-end), linked by a small peptide (DFGGGGG), were expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TAK1 gene accession number is NM_003188; TAB1 is NM_006116.

Gene Aliases

TAK1: MAP3K7, TGF1a; TAB1: MAP3K7IP1, 3'-Tab1, MGC57664

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

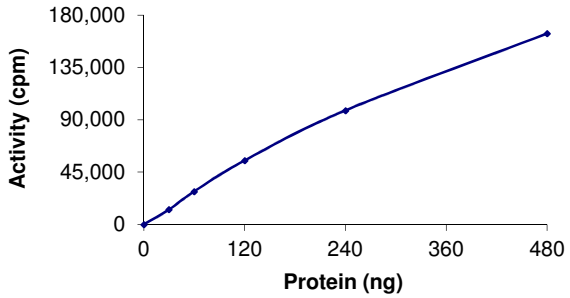
Scientific Background

TAK1 is a serine/threonine protein kinase that mediates signaling by TGFβ and morphogenetic protein (BMP) (1). In response to IL-1, TAK1 forms a kinase complex with TAB1 and this complex is required for the activation of nuclear factor kappa B (NfκB). TAK1 can also activate MAPK8/JNK and MAP2K4/MKK4 and thus play a role in the cell response to environmental stress. Tak1 is essential for thymocyte development and activation and deletion of TAK1 prevents maturation of single-positive thymocytes displaying CD4 or CD8 (2). Thymocytes lacking TAK1 fail to activate NfκB and JNK and are prone to apoptosis upon stimulation.

References

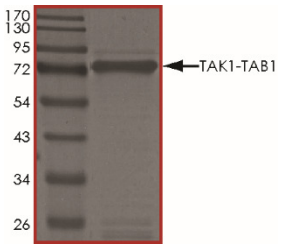
1. Yamaguchi, K. et al: Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science 270: 2008-2011, 1995.
2. Liu, H.-H. et al: Essential role of TAK1 in thymocyte development and activation. Proc. Nat. Acad. Sci. 103: 11677-11682, 2006.

Specific Activity



The specific activity of TAK1-TAB1 was determined to be **12 nmol /min/mg** as per activity assay protocol.

Purity



The purity of TAK1-TAB1 was determined to be **>95%** by densitometry.
Approx. MW **~74kDa**.

TAK1-TAB1, Active

Human fusion recombinant protein expressed in Sf9 cells

Catalog #	M15-13G
Specific Activity	12 nmol/min/mg
Specific Lot #	T1064-3
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M15-13G)

Active TAK1-TAB1 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active TAK1-TAB1 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active TAK1-TAB1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active TAK1-TAB1 (Catalog #M15-13G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5µl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [^{33}P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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