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Certificate of Analysis

TAK1-TAB1 fusion, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-600, 14-600-K, 14-600M
Parent Lot # D13DP016N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, human TAK1 amino acids 1–303, fused to human TAB1 amino acids 437–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 88% by SDS-PAGE and Coomassie blue staining. MW = 42.8kDa.

Specific Activity (Parent lot# D13DP016N): 72U/mg, where one unit of TAK1-TAB fusion activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.231mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

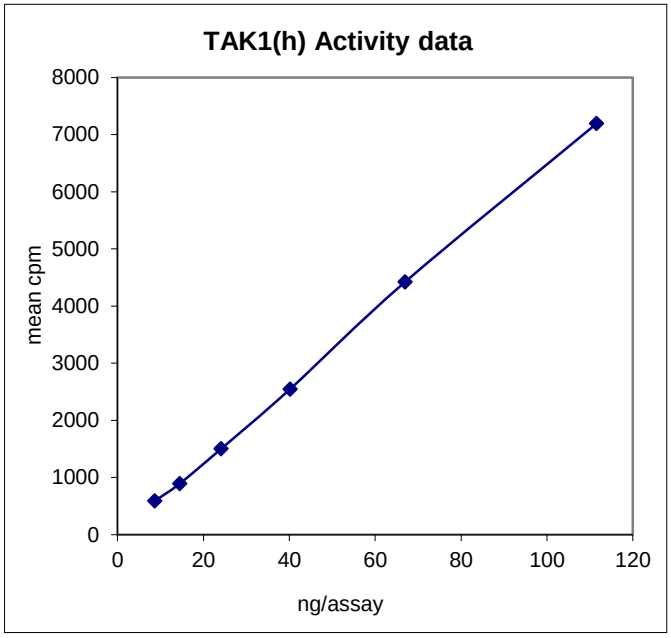
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

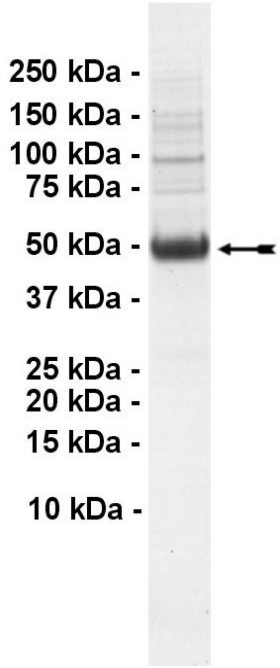
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 9–112ng of this lot of enzyme phosphorylated 2mg/ml casein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as TAK1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active TAK1-TAB fusion.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Casein:** Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
3. **TAK1-TAB fusion, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–112ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer to wells.
2. Add 2.5µl of **casein**.
3. Add **2.5µl (9–112ng) TAK1-TAB fusion, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl pf 30% phosphoric acid.

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TAK1-TAB fusion Sequence Information

Protein Human TAK1

Tags N-terminal 6His

Native sequence M9 of the recombinant protein is equivalent to M1 of human TAK1. Q319 of the recombinant protein is equivalent to Q437 of human TAB1 (GenBank NM_006116)

Accession number GenBank NM_003188

Recombinant TAK1-TAB fusion amino acid sequence:

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  1 MGHHHHHHMS TASAASSSSS SSAGEMIEAP SQVLNFEEID YKEIEVEEVV GRGAFGVVCK
 61 AKWRAKDVAI KQIESESERK AFIVELRQLS RVNHPNIVKL YGACLNPVCL VMEYAEGGSL
121 YNVLHGAEPL PYYTAAHAMS WCLQCSQGVA YLHSMQPKAL IHRDLKPPNL LLVAGGTVLK
181 ICDFGTACDI QTHMTNNKGS AAWMAPEVFE GSNYSEKCDV FSWGIILWEV ITRRKPFDEI
241 GGPAFRIMWA VHNGTRPPLI KNLPKPIESL MTRCWSKDPS QRPSMEEIVK IMTHLMRYFP
301 GADEPLQYPC QEFGGGGGQS PTLTLQSTNT HTQSSSSSSD GGLFRSRPAH SLPPGEDGRV
361 EPYVDFAEFY RLWSVDHGEQ SVVTAP
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Recombinant TAK1-TAB fusion nucleotide sequence:

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   1 atgggtcatc accatcacca tcacatgtct acagcctctg ccgcctcctc ctcctcctcg
  61 tcttcggccg gtgagatgat cgaagcccct tcccaggtcc tcaactttga agagatcgac
 121 tacaaggaga tcgaggtgga agaggttgtt ggaagaggag cctttggagt tgtttgcaaa
 181 gctaagtgga gagcaaaaga tgttgctatt aaacaaatag aaagtgaatc tgagaggaaa
 241 gcgtttattg tagagcttcg gcagttatcc cgtgtgaacc atcctaatat tgtaaagctt
 301 tatggagcct gcttgaatcc agtgtgtctt gtgatggaat atgctgaagg gggctcttta
 361 tataatgtgc tgcatggtgc tgaaccattg ccatattata ctgctgccca cgcaatgagt
 421 tggtgtttac agtgttccca aggagtggct tatcttcaca gcatgcaacc caaagcgcta
 481 attcacaggg acctgaaacc accaaactta ctgctggttg caggggggac agttctaaaa
 541 atttgtgatt ttggtacagc ctgtgacatt cagacacaca tgaccaataa caaggggagt
 601 gctgcttgga tggcacctga agtttttgaa ggtagtaatt acagtgaaaa atgtgacgtc
 661 ttcagctggg gtattattct ttgggaagtg ataacgcgtc ggaaaccctt tgatgagatt
 721 ggtggcccag ctttccgaat catgtgggct gttcataatg gtactcgacc accactgata
 781 aaaaatttac ctaagcccat tgagagcctg atgactcgtt gttggtctaa agatccttcc
 841 cagcgccctt caatggagga aattgtgaaa ataatgactc acttgatgcg gtactttcca
 901 ggagcagatg agccattaca gtatccttgt caggaattcg gaggaggagg aggacaaagc
 961 ccgaccttaa ccctgcagtc caccaacacg cacacgcaga gcagcagctc cagctctgac
1021 ggaggcctct tccgctcccg gcccgcccac tcgctcccgc ctggcgagga cggtcgtgtt
1081 gagccctatg tggactttgc tgagttttac cgcctctgga gcgtggacca tggcgagcag
1141 agcgtggtga cagcaccgta g
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