

Certificate of Analysis

TAO1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-749, 14-749-K, 14-749M

Parent Lot # D9BN023U

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 TAO1 amino acids 1–327. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 95.5% by SDS-PAGE and Coomassie blue staining. MW = 41kDa

Specific Activity (Parent lot# D9BN023U): 1435U/mg, where one unit of TAO1 activity is defined as 1nmol phosphate incorporated into 0.8mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.438mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

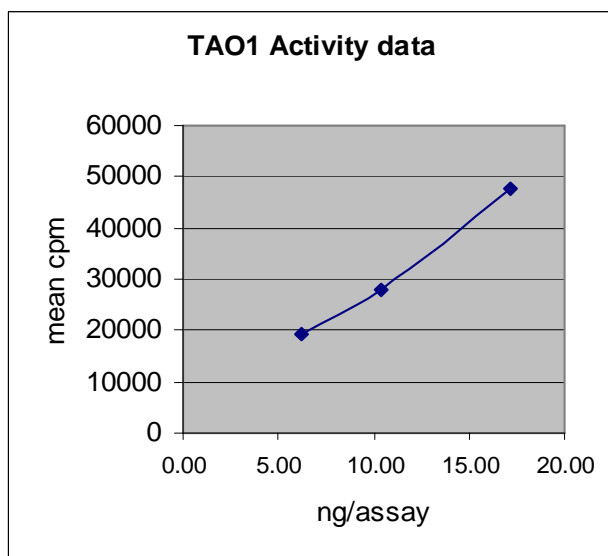
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

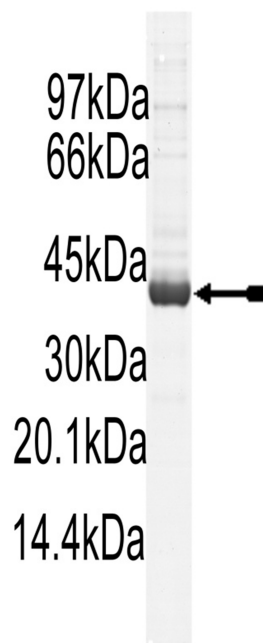
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6.24–17.19ng of this lot of enzyme phosphorylated 0.8mg/ml myelin basic protein 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 identity as TAO1 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 TAO1, active.

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Substrate: Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.8mg/ml. Make up a 8.0mg/ml stock. Use 2.5µl of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 50mM. Make a 3M stock. Add 0.42µl of stock per assay point.
4. **TAO1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.24–17.19ng per assay point.
5. **[γ -³³P] ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein**.
3. Add **2.5µl (6.24–17.19ng) TAO1, active**.
4. Add 0.42µl of 3M NaCl.
5. Add 4.58µl of dH₂O.
6. Add 10µl of diluted [γ -³³P] ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

Certificate of Analysis

TAO1 Sequence Information

<u>Protein</u>	Human TAO1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of recombinant sequence = M1 of native human TAO1
<u>Accession number</u>	GenBank NM_020791

Recombinant TAO1 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MPSTNRAGSL KDPEIAELFF KEDPEKLF
61 LREIGHGSFG AVYFARDVRT NEVVAIKKMS YSGKQSTEKW QDIIKEVKFL QRIKHPNSIE
121 YKGCYLREHT AWLVMEYCLG SASDLLLEVHK KPLQEVEIAA ITHGALQGLA YLHSHTMIHR
181 DIKAGNILLT EPGQVKLADF GSASMASPAN SFVGTPTYWMA PEVILAMDEG QYDGKVDVWS
241 LGITCIELAE RKPPLFNMNA MSALYHIAQN ESPTLQSNEW SDYFRNFVDS CLQKIPQDRP
301 TSEELLKHIF VLRERPETVL IDLIQRTKDA VRELDNLQYR KMKKLLFQEA HNGPAVE

```

Recombinant TAO1 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgccatcaa ctaacagagc aggcagcctg
121 aaggaccctg aaattgcaga gctcttcttc aaagaagatc cagagaagct cttcacagat
181 ctcagagaaa ttggccatgg aagctttgga gcagtgtatt ttgcacgaga tgtgctgacc
241 aatgaagtgg tggccatcaa gaaaatgtct tatagtggaa agcagtctac tgagaaatgg
301 caggatatta ttaaggaagt caagtttcta caaagaataa aacatcccaa cagtatagaa
361 taciaaaggct gttatttacg tgaacacaca gcatggcttg taatggaata ttgttttagga
421 tctgcttcgg atttactaga agttcacaaa aagccattac aagaagtgga aatagcagca
481 attacacatg gtgctcttca gggattagcc tacttacatt ctcatactat gattcataga
541 gatatacaag caggaaatat ccttctgaca gaaccaggcc aggtgaaact tgctgacttt
601 ggctctgctt ccatggcatc acctgccaat tcctttgtgg gaacgccgta ttggatggcc
661 ccagaagtaa ttttagccat ggatgaagga caatatgatg gcaaagtaga tgtgtggtct
721 cttggaataa catgtattga actagcggaa aggaagcctc ctttatttaa tatgaatgca
781 atgagtgcct tatacacat agcccaaaat gaatccccta cactacagtc taatgaatgg
841 tctgattatt ttcgcaactt tgtagattct tgccccaga aaatccctca agatcgacct
901 acatcagagg aactttttaa gcacatattt gttcttcggg agcgccctga aaccgtgtta
961 atagatctca ttcagaggac aaaggatgca gtaagagagc tggacaatct gcagtatcga
1021 aagatgaaga aactcctttt ccaggaggca cataatggac cagcagtaga ataa

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.