

Certificate of Analysis

TAO3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-745, 14-745-K, 14-745M

Parent Lot # D7BN007U

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 TAO3 amino acids 1–411. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 87.4% by SDS-PAGE and Coomassie blue staining. MW = 50.2kDa

Specific Activity (Parent lot# D7BN007U): 1084U/mg, where one unit of TAO3, active 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: 2.788mg/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.

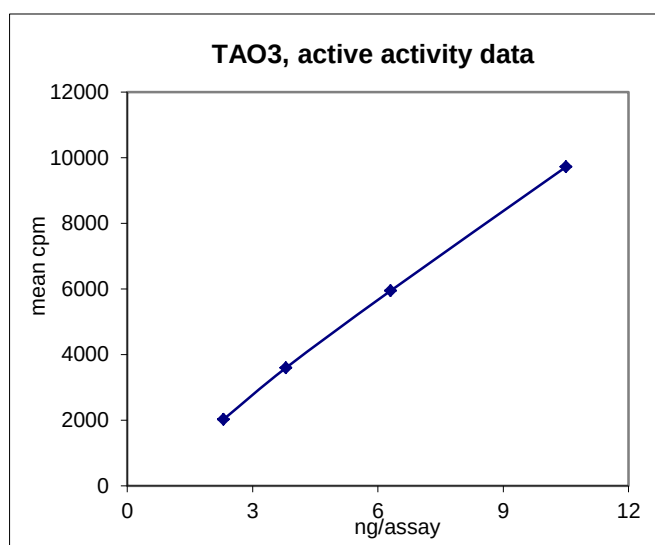
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

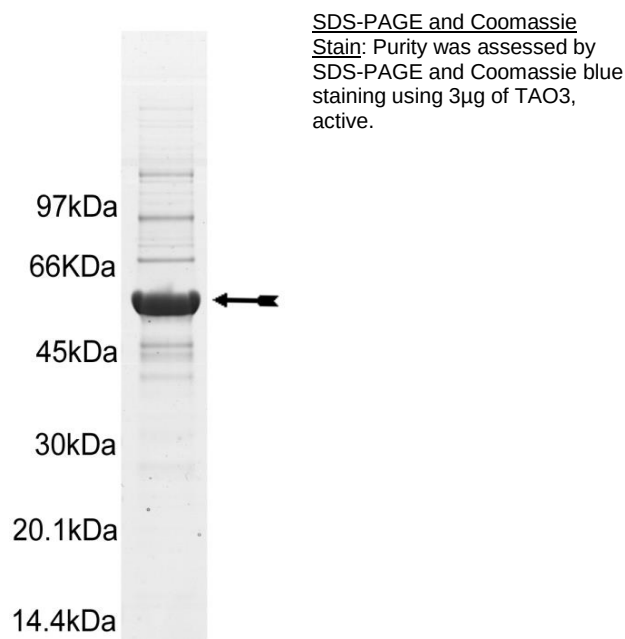
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.3–10.5ng 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 TAO3 with the translated sequence listed on page three.



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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 an 8.0mg/ml stock. Use 2.5µl of stock solution per assay point.
3. **NaCl:** Use at a final assay concentration of 200mM. Make a 3M stock. Add 1.67µl of stock per assay point.
4. **TAO3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 2.3–10.5ng 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 (2.3–10.5ng) TAO3, active**.
4. Add 1.67µl of 3M NaCl.
5. Add 3.33µl 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.

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TAO3 Sequence Information

<u>Protein</u>	Human TAO3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of recombinant sequence = M1 of native human TAO3
<u>Accession number</u>	GenBank NM_016281. The recombinant protein contains the amino acid substitution N47S with reference to NM-016281. This mutation is reported in GenBank BC002756 and BC7007185

Recombinant TAO3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MRKGVVKDPE IADLFYKDDP EELFIGLHEI
61 GHGSFGAVYF ATNAHTSEVV AIKKMSYSGK QTHEKWQDIL KEVKFLRQLK HPNTIEYKGC
121 YLKEHTAWLV MEYCLGSASD LLEVHKKPLQ EVEIAAITHG ALHGLAYLHS HALIHRDIKA
181 GNILLTEPGQ VKLADFGSAS MASPANSFVG TPYWMAPEVI LAMDEGQYDG KVDIWSLGIT
241 CIELAERKPP LFNMNAMSAL YHIAQNDSPT LQSNWTDSEF RRFVDYCLQK IPQERPTSAE
301 LLRHDFVRRD RPLRVLIDLI QRTKDAVREL DNLQYRKMKK ILFQETRNGP LNESQEDEED
361 SEHGTSLNRE MDSLGSNHSI PSMSVSTGSQ SSSVNSMQEV MDESSSELVM MHDDESTINS
421 SSSVVHKKDH VFIRDEAGHG D

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Recombinant TAO3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atgcgtaaag ggggtgctgaa ggaccagag
121 attgccgatc tattctacaa agatgatcct gaggaacttt ttattggttt gcatgaaatt
181 ggacatggaa gttttggagc agtttatttt gctacaaatg ctacaccagc tgagggtggtg
241 gcaattaaga agatgtccta tagtgggaag cagacccatg agaaatggca agatattctt
301 aaggaagtta aatttttacg acaattgaag catcctaata ctattgagta caaaggctgt
361 tacttgaaag aacacactgc ttggttggtg atggaatatt gcttaggctc agcctctgat
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481 gccttgcatg gactagccta cctacattct catgcattga ttcataaggga tattaagca
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1201 atggacgaga gcagttccga acttgtcatg atgcacgatg acgaaagcac aatcaattcc
1261 agctcctccg tcgtgcataa gaaagatcat gtattcataa gggatgaggc gggccacggc
1321 gattaa

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