

## Certificate of Analysis

### TAO2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-736, 14-736-K, 14-736M

Parent Lot # 2037021

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, TAO2, amino acids 1–320, expressed in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 66.5% by SDS-PAGE and Coomassie blue staining. MW = 40.1kDa.

**Specific Activity (Parent lot# 2037021):** 641U/mg, where one unit of TAO2, 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:** 0.751mg/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.

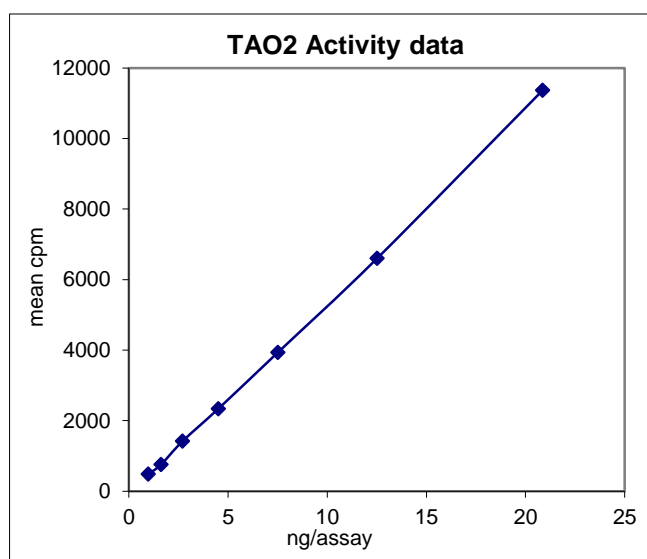
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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**

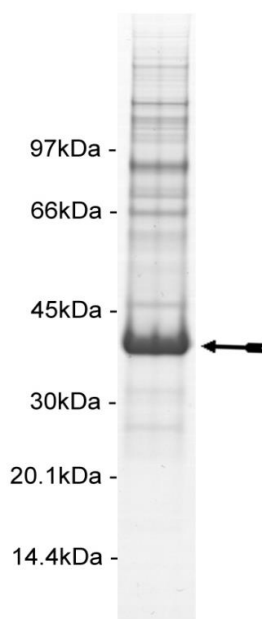
### Quality Control Testing

**Kinase Assay:** 1–21ng of this lot of enzyme phosphorylated 0.8mg/ml myelin basic protein (MBP) 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 TAO2 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 TAO2, active.



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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 per assay point.
3. **TAO2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–21ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]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 (1–21ng) TAO2, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>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 of 30% phosphoric acid.

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## TAO2 Sequence Information

<b><u>Protein</u></b>	Human TAO2
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	M31 of the recombinant sequence is equivalent to M1 of native human TAO2
<b><u>Accession number</u></b>	GenBank NM_016151

### ***Recombinant TAO2 amino acid sequence:***

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MPAGGRAGSL KDPDVAELFF KDDPEKLFSD
61 LREIGHGSFG AVYFARDVRN SEVVAIKKMS YSGKQSNEKW QDIIKEVRFL QKLRHPNTIQ
121 YRGCYLREHT AWLMEYCLG SASDLLEVHK KPLQEVEIAA VTHGALQGLA YLHSHNMIHR
181 DVKAGNILLS EPLVKLGDF GSASIMAPAN SFVGTPYWMA PEVILAMDEG QYDGKVDVWS
241 LGITCIELAE RKPLPFNMNA MSALYHIAQN ESPVLQSGHW SEYFRNFVDS CLQKIPQDRP
301 TSEVLLKHRF VLRERPPTVI MDLIQRTKDA VRELDNLQYR KMKKILFQEA

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### ***Recombinant TAO2 nucleotide sequence:***

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgccagctg ggggccgggc cgggagcctg
121 aaggaccagc atgtggctga gctcttcttc aaggatgacc cagaaaagct cttctctgac
181 ctccgggaaa ttggccatgg cagctttgga gccgtatact ttgcccggga tgtccggaat
241 agtgaggctg ttggccatcaa gaagatgtcc tacagtggga agcagtccaa tgagaaatgg
301 caagacatca tcaaggaggc gcggttctta cagaagctcc ggcatcccaa caccattcag
361 taccggggct gttacctgag ggagcacacg gcttggctgg taatggagta ttgcctgggc
421 tcagcttctg accttctaga agtgacaag aaaccccttc aggaggtaga gatcgcagct
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1021 aagatgaaga agatcctggt ccaaggagca taa

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