

Certificate of Analysis

TLK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-739, 14-739-K, 14-739M

Parent Lot # 1606090

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, 388-end, human TLK2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 71.1% by SDS-PAGE and Coomassie blue staining. MW = 48.8kDa.

Specific Activity (Parent lot# 1606090): 569U/mg, where one unit of TLK2, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.668mg/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.

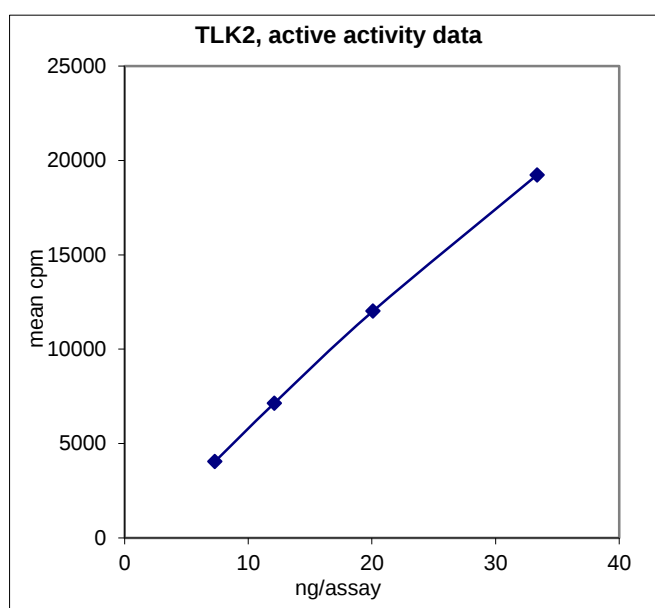
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

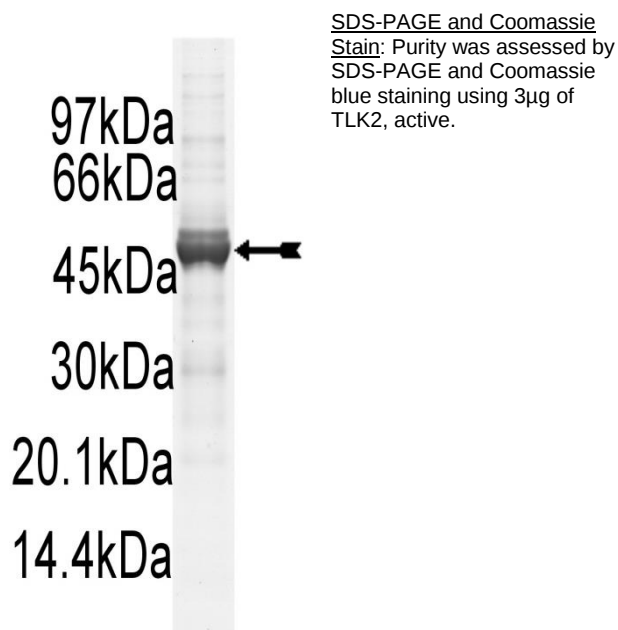
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 7–33ng 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 TLK2 with the translated native sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 X Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Casein:** Use at a final concentration of 2mg/ml. Make a 20mg/ml stock. Use 2.5µl per assay point.
3. **TLK2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–33ng per assay point.
4. **[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 **casein**.
3. Add **2.5µl (7–33ng) TLK2, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P] 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.

Certificate of Analysis

TLK2 Sequence Information

<u>Protein</u>	Human TLK2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	A37 of recombinant sequence is equivalent to A387 of native human TLK2
<u>Accession number</u>	GenBank BC044925

Recombinant TLK2 amino acid sequence:

```

1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQALGA HSLRLTLAE YHEQEEIFKL
61 RLGHLLKKEEA EIQAELERLE RVRNLHIREL KRIHNEDNSQ FKDHPTLNDR YLLLHLGRG
121 GFSEVYKAFD LTEQRYVAVK IHQLKNWRD EKKENYHKHA CREYRIHKEL DHPRIVKLYD
181 YFSLDTSDFC TVLEYCEGND LDFYLKQHKL MSEKEARSII MQIVNALKYL NEIKPPIIHY
241 DLKPGNILLV NGTACGEIKI TDFGLSKIMD DDSYNSVDGM ELTSQGAGTY WYLPPECFVV
301 GKEPPKISNK VDVWSVGVIF YQCLYGRKPF GHNQSQQDIL QENTILKATE VQFPKPVVT
361 PEAKAFIRRC LAYRKEDRID VQQLACDPYL LPHIRKSVST SSPAGAAIAS TSGASNNSSS
421 N

```

Recombinant TLK2 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaagc cttaggagcc
121 cacagtttac ttaggttaac gtttagcaga taccatgaac aagaagaaat cttcaaactc
181 agattaggct atcttaaaaa ggagggaagc gagatccagg cagagctgga gagactagaa
241 agggtagtaa atctacatat caggggaacta aaaaggatac ataataga taattcacaa
301 tttaaagatc atccaacgct aaatgacaga ttttgttgt tacatctttt gggttagagga
361 gggttcagtg aagtttaca ggcatctgat ctaacagagc aaagatacgt agctgtgaaa
421 attcaccagt taaataaaaa ctggagagat gagaataagg agaattacca caagcatgca
481 ttaggggaat accggattca taaagagctg gatcatccca gaatagttaa gctgtatgat
541 tacttttcac tggatactga ctctgtttgt acagtattag aatactgtga gggaaatgat
601 ctggacttct acctgaaaca gcacaaatta atgtcggaga aagaggcccg gtccattatc
661 atgcagattg tgaatgcttt aaagtactta aatgaaataa aacctcccat catacactat
721 gacctcaaac caggttaatat tcttttagta aatggtacag cgtgtggaga gataaaaatt
781 acagattttg gtctttcgaa gatcatggat gatgatagct acaattcagt ggatggcatg
841 gagctaacat cacaagggtg tggtacttat tggattttac caccagagtg ttttgtggtt
901 gggaaagaac caccaaagat ctcaataaaa gttgatgtgt ggtcgggtgg tgtgatcttc
961 tatcagtgct tttatggaag gaagcctttt ggccataacc agtctcagca agacatccta
1021 caagagaata cgattcttaa agctactgaa gtgcagttcc cgccaaagcc agtagtaaca
1081 cctgaagcaa aggcgtttat tcgacgatgc ttggcctacc gaaaggagga ccgcattgat
1141 gtccagcagc tggcctgtga tccctacttg ttgcctcaca tccgaaagtc agtctctaca
1201 agtagccctg ctggagctgc tattgcatca acctctgggg cgtccaataa cagttcttct
1261 aattga

```

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