

Certificate of Analysis

TSSK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-021, 15-021-K, 15-021M

Parent Lot # 194981

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

Product Description: C-terminal 6His-tagged, recombinant, human TSSK3 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by size exclusion chromatography.

Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 31kDa.

Specific Activity (Parent lot# 194981): 729U/mg, where one unit of TSSK3 activity is defined as 1nmol phosphate incorporated into 100µM AMARAASAAALARRR per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.42mg/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.

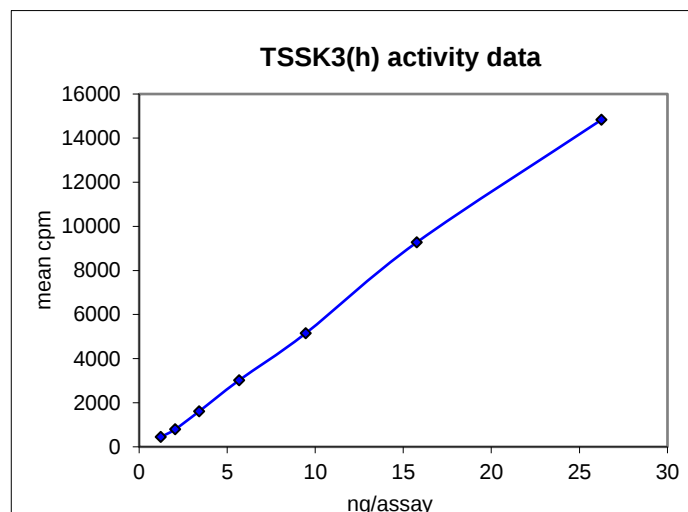
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

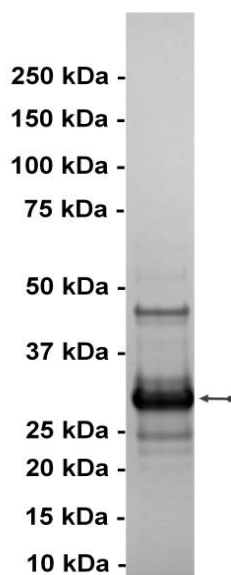
**FOR IN VITRO RESEARCH USE ONLY
 NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.2–26.3ng of this lot of enzyme phosphorylated 100µM AMARAASAAALARRR 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 TSSK3 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 TSSK3, active.

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Kinase Assay Protocol

Stock Solutions:

- 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- AMARAASAAALARRR:** Use at a final assay concentration of 100µM. Prepare a 1mM stock and add 2.5µl of stock per assay point.
- TSSK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–26.3ng per assay point.
- [γ -³³P]ATP:** 2.5 x MgAc/[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):

- Add 5µl of 5 x reaction buffer per assay to wells.
- Add 2.5µl of AMARAASAAALARRR
- Add 2.5µl (**1.2-26.3ng**) of **TSSK3 active**.
- Add 5µl of dH₂O.
- Add 10µl of diluted [γ -³³P]ATP mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5µl of 3% phosphoric acid.
- Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the dried filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
- 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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TSSK3, active Sequence Information

<u>Protein</u>	Human TSSK3
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human TSSK3
<u>Accession number</u>	GenBank NM_052841.2

Recombinant TSSK3 amino acid sequence:

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1 MEDFLLSNGY QLGKTIGEGT YSKVKEAFSK KHQRKVAIKV IDKMGGPEEF IQRFLPRELQ
61 IVRTL DHKNI IQVYEMLESA DGKICLV MEL AEGGDV FDCV LGGPLPESR AKALFRQ MVE
121 AIRYCHGCGV AHRDLKCENA LLQGFNLKLT DFGFAKVL PK SHRELSQTFC GSTAYAAPEV
181 LQGI PHDSK GDVWSMGVVL YVMLCASLPF DTDIPKMLW QQQKGV SFPT HLSISAD CQD
241 LLKRLL EPDM ILRPSIEEVS WHPWLASTHH HHHH
  
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Recombinant TSSK3 nucleotide sequence:

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1 atggaggact ttctgctctc caatgggtac cagctgggca agaccattgg ggaagggacc
61 tactcaaaag tcaaagaagc attttccaaa aaacacccaa gaaaagtggc aattaaagtt
121 atagacaaga tgggagggcc agaagagttt atccagagat tcctccctcg ggagctccaa
181 atcgctccgta ccctggacca caagaacatc atccaggtgt atgagatgct ggagtctgcc
241 gacgggaaaa tctgcctggt gatggagctc gctgagggag gggatgtctt tgactgcgtg
301 ctgaatgggg ggccactgcc tgaaagccgg gccaaaggccc tcttccgtca gatggttgag
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421 ttgttgacagg gcttcaacct gaagctgact gactttggct ttgccaaggt gttgcccaag
481 tcacaccggg agctgagcca gaccttctgc ggcagtagac cctatgctgc ccccgagggtg
541 ctgcagggca ttccccacga tagcaaaaaa ggtgatgtct ggagcatggg tgtggtcctg
601 tatgtcatgc tctgtgccag cctacctttt gacgacacag acatccccaa gatgctgtgg
661 cagcagcaga agggggtgtc cttccccact catctgagca tctcggccga ttgccaggac
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781 tggcatccat ggctagcaag cactcatcac catcaccatc actaa
  
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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.