

Certificate of Analysis

TSSK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-017, 15-017-K, 15-017M

Parent Lot # 197188

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 GST tagged and C-terminal 6His tagged, recombinant, human TSSK4 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity and size exclusion chromatography.

Purity 46% by SDS-PAGE and Coomassie blue staining. MW = 65kDa.

Specific Activity (Parent lot# 197188): 198U/mg, where one unit of TSSK4 activity is defined as 1nmol phosphate incorporated into 250µM KKLNRTLFAEPG per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.38mg/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.

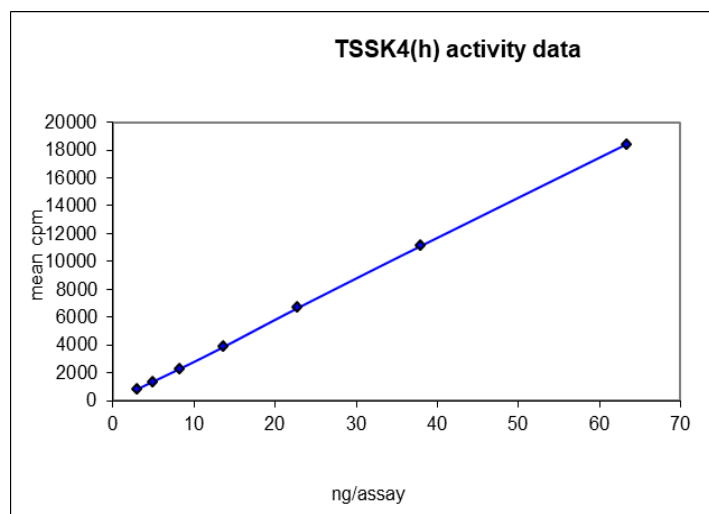
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

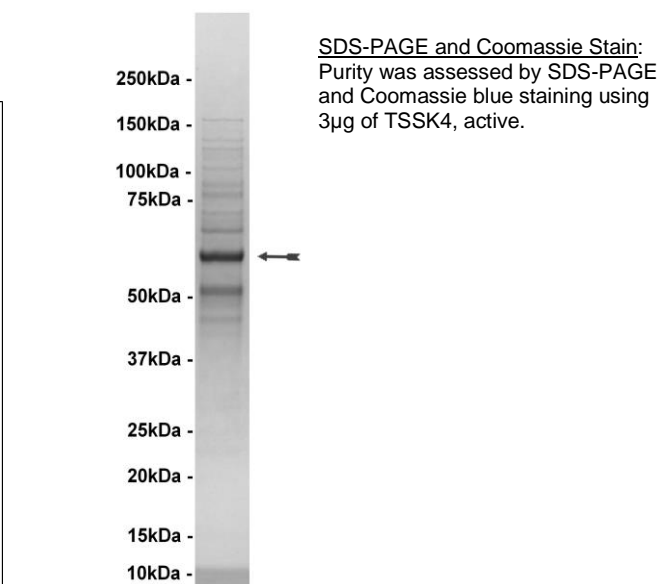
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.0–63.3ng of this lot of enzyme phosphorylated 250µM KKLNRTLFAEPG 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 TSSK4 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. KKLNRTLSFAEPG:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
- 3. TSSK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.0–63.3ng per assay point.
- 4. [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):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KKLNRTLSFAEPG.
3. Add **2.5 μ l (3.0–63.3ng) TSSK4, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 dried 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 1 μ l of 30% phosphoric acid.

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TSSK4, active Sequence Information

<u>Protein</u>	Human TSSK4
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	M231 of the recombinant protein is equivalent to M1 of human TSSK4
<u>Accession number</u>	GenBank AY461663.1

Recombinant TSSK4 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPFE  MGKGDVLEAA
241 PTTTAYHSLM  DEGYEVGKA  IGHGSYGSVY  EAFYTKQKVM  VAVKIIISKK  ASDDYLNKFL
301 PREIQVMKVL  RHKYLINFYR  AiestsrVYI  ILELAQGGDV  LEWIRQYGAC  SEPLAGKWFS
361 QLTLGIAYLH  SKSIVHRDLK  LENLLLDKWE  NVKISDFGFA  KMVPSNQPVG  CSPSYRQVNC
421 FSHLSQTYCG  SFAYACPEIL  RGLPYNPFLS  DTWSMGVILY  TLVVAHLPDF  DTNLKLLRE
481 TQKEVTFPAN  HTISQECKNL  ILQMLRQATK  RATILDI IKD  SWVLKFQPEQ  PTHEIRLLEA
541 MCQLHNTTKQ  HQSLQITTHH  HHHH

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Recombinant TSSK4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1441  actcagaagg  aggtcacttt  cccagctaac  cataccatct  cccaggagtg  caagaacctg
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1621 atgtgccagc tccacaacac cactaaacag caccaatcct tgcaaattac gacccatcat  
1681 caccatcacc attaa
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Reviewed and approved by site quality representative.

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