

Certificate of Analysis

TSSK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-670, 14-670-K, 14-670M

Parent Lot # 0701048785

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, full length TSSK1, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 69% by SDS-PAGE and Coomassie blue staining. MW = 46kDa.

Specific Activity (Parent lot# 0701048785): 526/mg, where one unit of TSSK1, active activity is defined as 1nmol phosphate incorporated into 300µM (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.98mg/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.

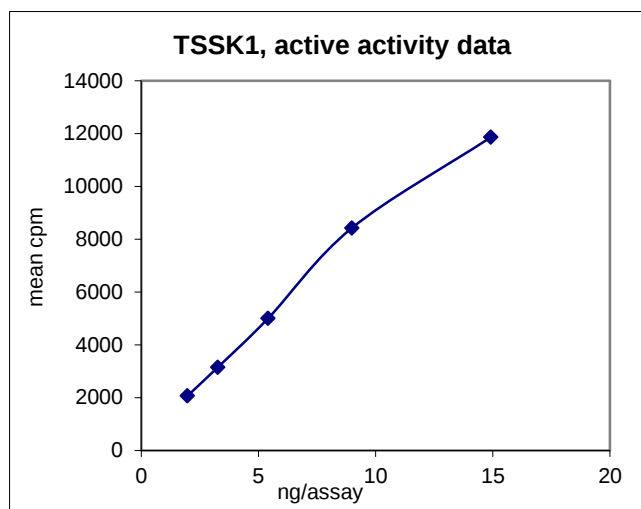
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

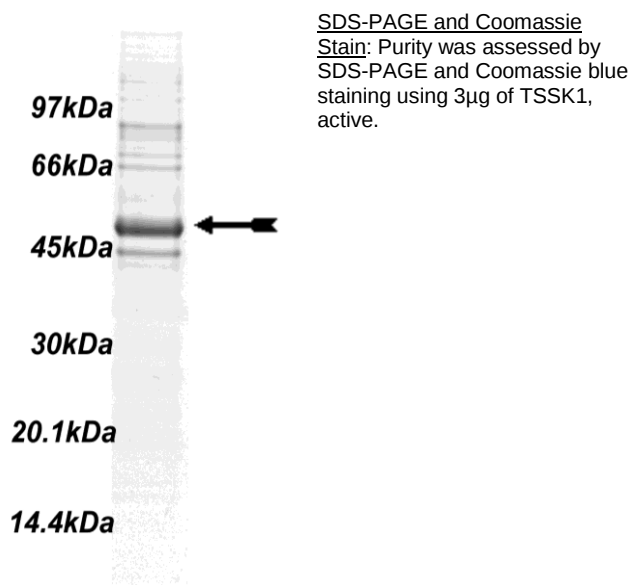
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–15ng of this lot of enzyme phosphorylated 300µM (KKKVSRSGLYRSPSPENLNRPR) 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 TSSK1 with the translated native 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. **(KKKVSRSGLYRSPSPENLNRP)**: Use at a final concentration of 300µM. Make a 3mM stock. Add 2.5µl of stock per assay point.
3. **TSSK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–15ng 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 300µM **(KKKVSRSGLYRSPSPENLNRP)**.
3. Add **2.5µl (2–15ng) TSSK1, 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.

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TSSK1 Sequence Information

<u>Protein</u>	Human TSSK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human TSSK1
<u>Accession number</u>	GenBank NM_032028

Recombinant TSSK1 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MDDAAVLKRR GYLLGINLGE GSYAKVKSAY
61 SERLKFNVAI KIIDRKKAPA DFLEKFLPRE IEILAMLNHC SIIKTYEIFE TSHGKVYIVM
121 ELAVQGDLLI LIKTRGALHE DEARKKFHQL SLAIKYCHDL DVVHRDLKCD NLLLDKDFNI
181 KLSDFSFSKR CLRDDSGRMA LSKTFCGSPA YAAPEVLQGI PYQPKVYDIW SLGVILYIMV
241 CGSMPYDDSN IKKMLRIQKE HRVNFPRSKH LTGECKDLIY HMLQPDVNRRL HIDEILSHC
301 WMQPKARGSP SVAINKEGES SRGTEPLWTP EPGSDKKSAT KLEPEGEAQP QAQPETKPEG
361 TAMQMSRQSE ILGFPSKPST METEEGPPQQ PPETRAQ

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Recombinant TSSK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atggatgacg ctgctgtcct caagcgacga
121 ggctacctcc tggggataaa ttttaggagag ggctcctatg caaaagtaaa atctgcttac
181 tctgagcgcc tgaagttcaa tgtggcgatc aagatcatcg accgcaagaa ggcccccgca
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