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Certificate of Analysis

TSSK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-632, 14-632-K, 14-632M
Parent Lot # D8KN010U

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, full length, human TSSK2, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 44.8kDa.

Specific Activity (Parent lot# D8KN010U): 1552U/mg, where one unit of TSSk2, active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.718mg/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.

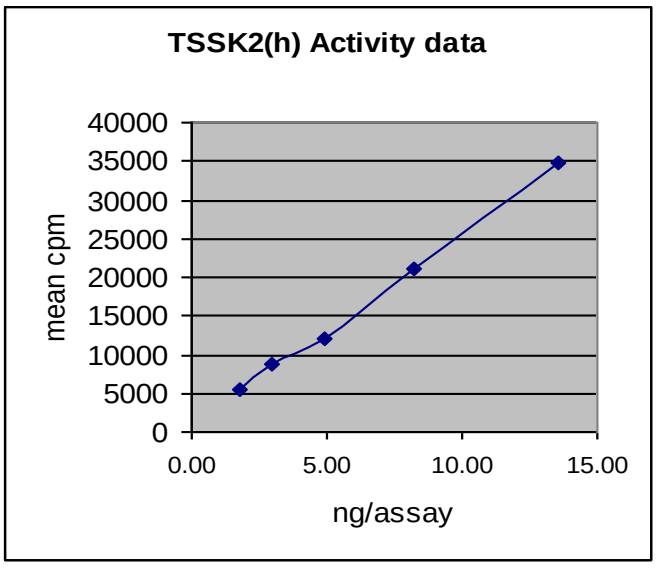
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

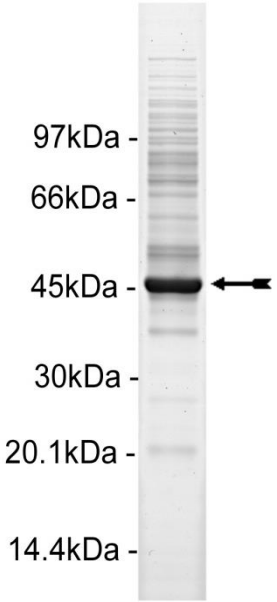
Quality Control Testing

Kinase Assay: 1.8–13.6ng of this lot of enzyme phosphorylated 0.1mM CHKtide 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 TSSK2 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of TSSK2, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **CHKtide:** Use at a final assay concentration of 100µM. Prepare a 1mM stock and add 2.5µl of stock per assay point.
3. **TSSK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.8–13.6ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}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 **CHKtide**.
3. Add **2.5µl (1.8–13.6ng) TSSK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}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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TSSK2 Sequence Information

Protein TSSK2

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human TSSK2

Accession number GenBank NM_053006

Recombinant TSSK2 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDDATVLRKK GYIVGINLGK GSYAKVKSAY
  61 SERLKFNVAV KIIDRKKTPT DFVERFLPRE MDILATVNHG SIIKTYEIFE TSDGRIYIIM
 121 ELGVQGDLLE FIKCQGALHE DVARKMFRQL SSAVKYCHDL DIVHRDLKCE NLLLDKDFNI
 181 KLSDFGFSKR CLRDSNGRII LSKTFCGSAA YAAPEVLQSI PYQPKVYDIW SLGVILYIMV
 241 CGSMPYDDSD IRKMLRIQKE HRVDFPRSKN LTCECKDLIY RMLQPDVSQR LHIDEILSHS
 301 WLQPPKPKAT SSASFKREGE GKYRAECKLD TKTDLRPDHR PDHKLGAKTQ HRLLVVPENE
 361 NRMEDRLAET SRAKDHHISG AEVGKAST
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Recombinant TSSK2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggacgatg ccacagtcct aaggaagaag
 121 ggttacatcg taggcatcaa tcttggcaag ggttcctacg caaaagtcaa atctgcctac
 181 tctgagcgcc tcaagttcaa tgtggctgtc aagatcatcg accgcaagaa aacacctact
 241 gactttgtgg agagattcct tcctcgggag atggacatcc tggcaactgt caaccacggc
 301 tccatcatca agacttacga gatctttgag acctctgacg gacggatcta catcatcatg
 361 gagcttggcg tccagggcga cctcctcgag ttcatcaagt gccagggagc cctgcatgag
 421 gacgtggcac gcaagatgtt ccgacagctc tcctccgccg tcaagtactg ccacgacctg
 481 gacatcgtcc accgggacct caagtgcgag aaccttctcc tcgacaagga cttcaacatc
 541 aagctgtctg actttggctt ctccaagcgc tgcctgcggg acagcaatgg gcgcatcatc
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 661 ccctaccagc ccaaggtgta tgacatctgg agcctgggcg tgatcctgta catcatggtc
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1081 aacaggatgg aggacaggct ggccgagacc tccagggcca aagaccatca catctccgga
1141 gctgaggtgg ggaaagcaag cacctag
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Reviewed and approved by site quality representative.