

Certificate of Analysis

MSSK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-665, 14-665-K, 14-665M

Parent Lot # 1606078

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 MSSK1, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 67% by SDS-PAGE and Coomassie blue staining. MW = 65.7kDa.

Specific Activity (Parent lot# 1606078): 116U/mg, where one unit of MSSK1 activity is defined as 1nmol phosphate incorporated into 50µM PKCtide (ERM₁PRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.484mg/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.

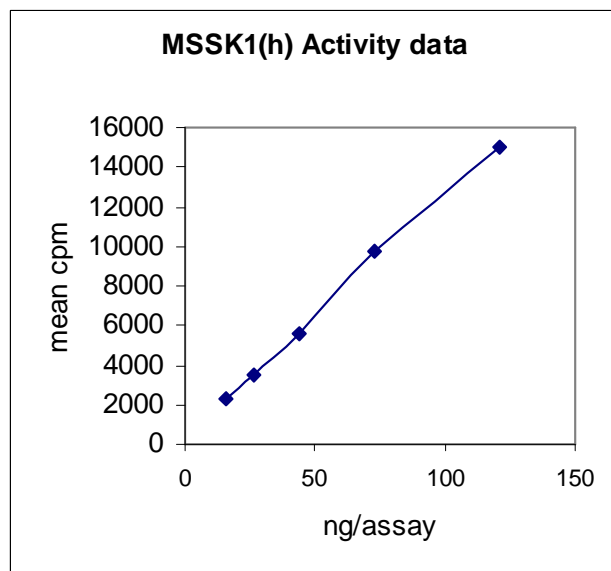
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

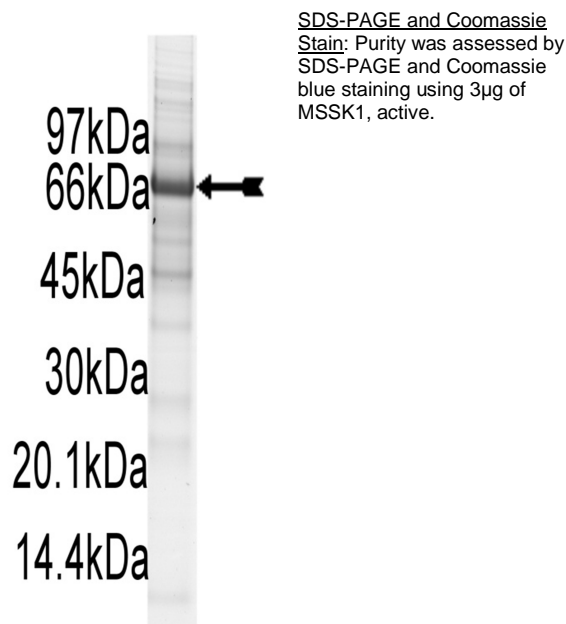
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 16–121ng of this lot of enzyme phosphorylated 50µM PKCtide 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 MSSK1 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. **PKCtide:** Use at a final assay concentration of 50 μ M. Make up a 500 μ M stock. Add 2.5 μ l of stock per assay point
3. **MSSK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 16–121ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PKCtide (ERM RPRKRQGSVRRRV)**.
3. Add **2.5 μ l (16–121ng) MSSK1, 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 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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MSSK1 Sequence Information

<u>Protein</u>	Human MSSK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human MSSK1
<u>Accession number</u>	GenBank NM_014370. The recombinant protein contains an insertion of 33 amino acid residues after G348 of the amino acid sequence shown below (equivalent to G318 of the native protein sequence). This conflict is reported in GenBank BX282601 and CD106669.

Recombinant MSSK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MSASTGGGGD SGGSGGSSSS SQASCGPESS
61 GSELALATPV PQMLQGLLGS DDEEQEDPKD YCKGGYHPVK IGDVFNGRYH VVRKLGWGHF
121 STVWLCWDIQ RKRFFALKVV KSAGHYTETA VDEIKLLKCV RSDPSDPKR ETIVQLIDDF
181 RISGVNGVHV CMVLEVLGHQ LLKWIISNY QGLPVCVKV IVRQVLHGLD YLHTKCKIIH
241 TDIKPENILL CVGDAYIRRL AAEATEWQQA GAPPPRSIV STAPQEVLTG KLSKNKRKKM
301 RRRKQKQKRL LEERLRLQR LEAMEAATQA EDSGLRLDGG SGSTSSSGCH PGGARAGPSP
361 ASSSPAPGGG RSLSAGSQTG GFSGLFSPA SCSILSGSSN QRETGGLLSP STPFASNLL
421 VNPLEPQNAD KIKIKIADLG NACVWHKFT EDIQTRQYRA VEVLIGAEYG PPADIWSTAC
481 MAFELATGDY LFEPHSGEDY SRDEDHIAHI VELLGDIPPA FALSGRYSRE FFNRRGELRH
541 IHNLKHWGLY EVLMEKYEWP LEQATQFSAF LLPMMEYIPE KRASAADCLQ HPWLNP

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Recombinant MSSK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atgagcgcca gcacgggcgg tgggtggggac
121 agcggcggca gcgggcggcag tagcagcagc tcacaggcct cctgcgggcc cgagtcctcg
181 ggctccgaac tagccctggc cacaccgggt cctcagatgc tcagggcct tctgggctcc
241 gacgacgagg aacaggaaga ccccaagac tactgcaagg gcggctacca ccctgtgaag
301 atcggcgacg tgttcaatgg gcggtaccac gtggtgcgca aactgggctg gggccacttc
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421 aagagtgcgg ggcattacac ggagacagct gtggatgaga tcaagctcct gaaatgtgtc
481 cgggacagcg accccagtga ccccaaaaga gagaccattg tccagctcat tgatgacttc
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661 atcgtgaggc aggtgctgca cggcctggac tacctccaca ccaagtgcaa gatcatccac
721 acggacatca agcccgagaa catcttgctg tgtgtggggg acgcttacat caggcgctg
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1261 gtgaaccccc tggagccca aatgcagat aagatcaaga tcaagatcg agacctggg
1321 aacgcctgct gggtgcacaa gcacttcacg gaagacatcc agactcggca gtaccgggccc
1381 gtcgagggtc tgatcggcgc cgaatacggc cccccggcag acatctggag cacagcctgc
1441 atggccttc agctggccac tggtgactac ctgttcgagc cgcattctgg agaagactac
1501 agtcgtgatg aggaccacat cgctcacata gtggagcttc tgggggacat cccccagcc
1561 ttcgccctct caggccgcta ttcccgggag ttcttcaacc ggagaggaga gctgcggcac

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1621 atccacaatc tcaagcactg gggcctgtac gaggtactca tggaaaagta cgagtggccc  
1681 ctagagcagg ccacacagtt cagcgccttt ctgctgccca tgatggagta catccccgaa  
1741 aagcgggcca gtgccgctga ctgcctccag caccctggc tcaacccta g
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Reviewed and approved by site quality representative.

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