

Certificate of Analysis

MSK1 (2-end), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-548, 14-548-K, 14-548M

Parent Lot # D8PN049U

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 MSK1 residues 2-end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose, activated using human MAPK2, and then re-purified using Ni²⁺/NTA agarose. Purity 93.3% by SDS-PAGE and Coomassie blue staining. MW = 94kDa.

Formulation: 2.043mg/ml of enzyme in 50mM Tris/HCl pH7.5, 50% glycerol, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

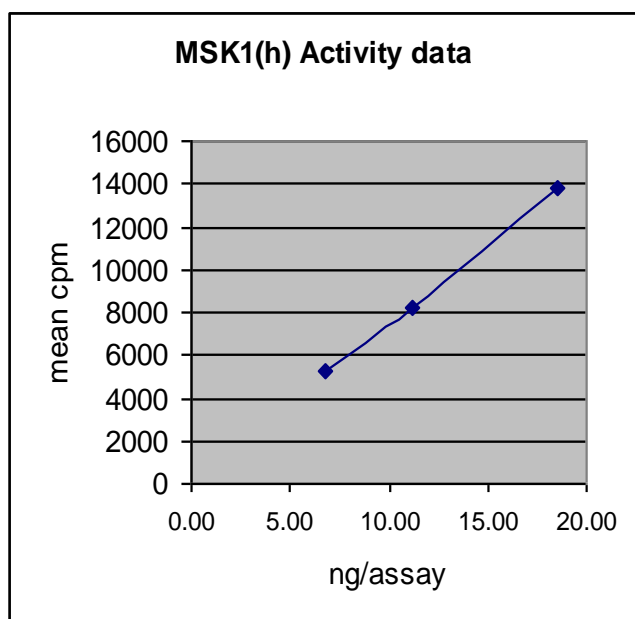
Specific Activity (Parent lot# D8PN049U): 404U/mg, where one unit of MSK1, active activity is defined as 1nmol phosphate incorporated into 30µM modified crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100µM.

Storage and Stability: On receipt of material store at -20°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.

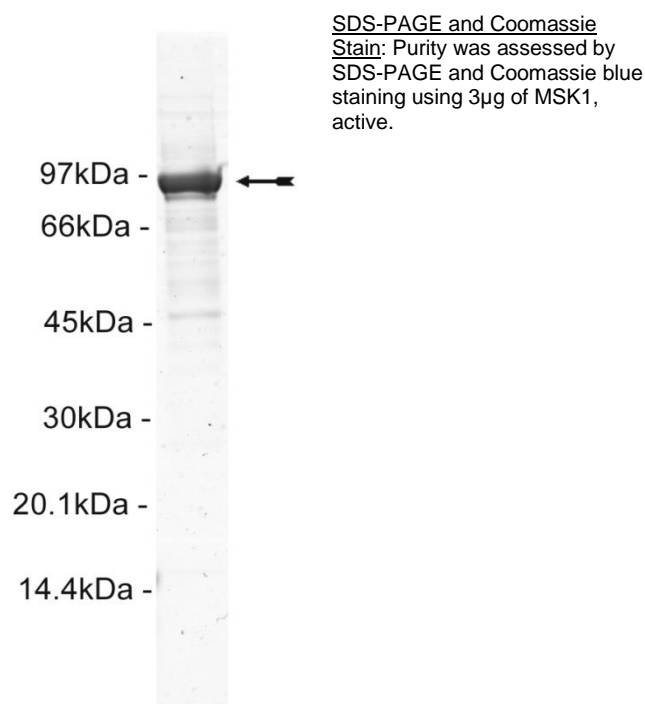
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6.7–18.5ng of this lot of enzyme phosphorylated 30µM modified crosstide 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 MSK1 with the translated sequence listed on pages three and four.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Modified Crosstide (GRPRTSSFAEGKK):** Use at a final assay concentration of 30 μ M. Prepare a 300 μ M stock and add 2.5 μ l of stock per assay point.
- 3. MSK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.7–18.5ng 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 **modified crosstide**.
3. Add **2.5 μ l (6.7–18.5ng) MSK1, 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 reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of the **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 50mM 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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MSK1 Sequence Information

Protein	human MSK1 (2-end)
Tags	N-terminal 6His
Native sequence	E32 of the recombinant protein is equivalent to E2 of human MSK1
Accession number	GenBank AF074393

Recombinant MSK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KEEEGSSSGG AAGTSADGGD GGEQLLTVKH
61 ELRTANLTGH AEKVGIENFE LLKVLGTGAY GKVFLVRKIS GHDTGKLYAM KVLKKATIVQ
121 KAKTTEHTRT ERQVLEHIRQ SPFLVTLHYA FQTETKLHLI LDYINGGELF THLSQRERFT
181 EHEVQIYVGE IVLALEHLHK LGIIYRDIKL ENILLDSNGH VVLTDFGLSK EFVADETERA
241 YSFCGTIEYM APDIVRGGDS GHDKAVDWSW LGVLMYELLT GASPFTVDGE KNSQAEISRR
301 ILKSEPPYPQ EMSALAKDLI QRLLMKDPKK RLGCGPRDAD EIKEHLFFQK INWDDLAACK
361 VPAPFKPVIR DELDVSNFAE EFTEMPTYS PAALPQSSEK LFGYGSFVAP SILFKRNAAV
421 IDPLQFHMGV ERPGVTNVAR SAMMKDSPFY QHYDLDLKDK PLGEGSFSIC RKCVMHKSQ
481 AFAVKIISKR MEANTQKEIT ALKLCGHPN IVKLHEVFHD QLHTFLVMEL LNGGELFERI
541 KKKKHFSETE ASYIMRKLVS AVSHMHDVGV VHRDLKPENL LFTDENDNLE IKIIDFGFAR
601 LKPPDNQPLK TPCFTLHYAA PELLNQNGYD ESCDLWSLGV ILYTMLSGQV PFQSHDRSLT
661 CTSAVEIMKK IKKGDFFSFE EAWKNVSQEA KDLIQGLLTV DPNKRLKMSG LRYNEWLQDG
721 SQLSSNPLMT PDILGSSGAA VHTCVKATFH AFNKYKREGF CLQNVDKAPL AKRRKMKKTS
781 TSJETRSSSS ESSHSSSSHS HGKTTPTKTL QPSNPADSNN PETLFQFSDS VA
    
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Recombinant MSK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaggaggagg aggggtggcag cagcggcggc
121 gccgcgggga ccagcgcgga cggcggcgac ggaggagagc agctcctcac tgtcaagcac
181 gagctgcgga ctgctaattt gacaggacat gctgagaagg tgggaataga aaattttgag
241 ctctgaagg tcctaggaac tggagcttat ggaaaagtat ttctagtctg taaaataagt
301 ggcatgata ctggaagct gtatgccatg aaagtttga aaaaggcaac aatcgttcaa
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481 ttagattata taaatggtgg tgaactttt actcatctt ctcaaagaga gcgtttcaca
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1261 atagaccctc ttcagtttca catgggagtt gaacgtcctg gaggtagaaa tgttgccagg
1321 agtgcaatga tgaaggactc tccattctat caacactatg acctagattt gaaggacaaa
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1441 gcttttgcag tcaaaataat cagcaaaagg atggaagcca atactcaaaa ggaaataaca
1501 gctctgaaac tctgtgaagg acacccaat attgtgaagt tgcagtaagt ttttcatgat
    
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1561 cagcttcaca cgtttctagt gatggaactt ctgaatggag gagaactggt tgagcgcatt
1621 aagaaaaaga agcacttcag tgagacggaa gccagctaca tcatgaggaa gcttgtttca
1681 gctgtaagcc acatgcatga tgttggagtg gtgcacaggg atctgaaacc tgagaattta
1741 ttgttcaccg atgaaaatga caatttgaa attaaaataa ttgattttgg atttgcacgg
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1861 ccagagctct tgaatcagaa cggctacgat gagtcctgtg acctgtggag cttgggcgtc
1921 atttgtaca caatgttgc aggacagggt cccttccaat ccatgaccg aagtttgacg
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2101 gatccaaaca aaaggcttaa aatgtctggc ttgaggtaca atgaatggct acaagatgga
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2221 gtgcatacct gtgtgaaagc aaccttcac gcctttaaca aatacaagag agaggggttt
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2341 accagtaccg agacgcgcag cagttccagt gagagtccc attcttcttc ctctcattct
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2461 ccggagacc tcttcagtt ctcggactca gtagcttag
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