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Certificate of Analysis

MSK1 (2–end), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-438, 14-438-K, 14-438M
Parent Lot # D8AN041U

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 Ni^{2+}/NTA agarose, activated using human MAPK2, and then re-purified using Ni^{2+}/NTA agarose. Purity 93.5% by SDS-PAGE and Coomassie blue staining. MW = 94.4kDa.

Specific Activity (Parent lot# D8AN041U): 734U/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.

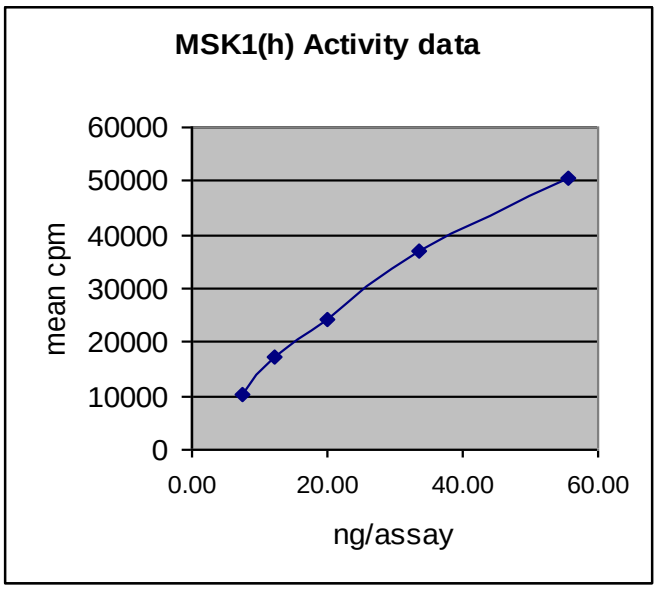
Formulation: 2.222mg/ml of enzyme in 50mM Tris/HCl pH7.5, 50% glycerol, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1 % 2-mercaptoethanol. Frozen solution.

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

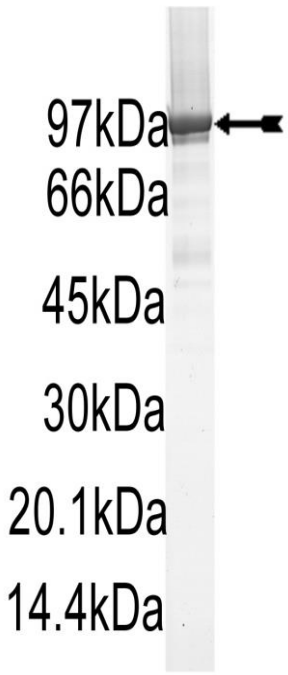
Quality Control Testing

Kinase Assay: 7–55ng 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 page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MSK1, active.



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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 30µM. Prepare a 300µM stock and add 2.5µl 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 7–55ng 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 **modified crosstide**.
3. Add **2.5µl (7–55ng) MSK1, 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 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 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 1µl of 30% phosphoric acid.

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MSK1 Sequence Information

Protein	human MSK1 (2–end)
Tags	*N*-terminal 6His
Native sequence	E39 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 YFQGAMGSAT MDYKDDDDKE EEGGSSGGAA GTSADGGDGG
  61 EQLLTVKHEL RTANLTGHAE KVGIENFELL KVLGTGAYGK VFLVRKISGH DTGKLYAMKV
 121 LKKATIVQKA KTTEHTRTER QVLEHIRQSP FLVTLHYAFQ TETKLHLILD YINGGELFTH
 181 LSQRERFTEH EVQIYVGEIV LALEHLHKLG IIYRDIKLEN ILLDSNGHVV LTDFGLSKEF
 241 VADETERAYS FCGTIEYMAP DIVRGGDSGH DKAVDWWSLG VLMYELLTGA SPFTVDGEKN
 301 SQAEISRRIL KSEPPYPQEM SALAKDLIQR LLMKDPKKRL GCGPRDADEI KEHLFFQKIN
 361 WDDLAAKKVP APFKPVIRDE LDVSNFAEEF TEMDPTYSPA ALPQSSEKLF QGYSFVAPSI
 421 LFKRNAAVID PLQFHMGVER PGVTNVARSA MMKDSPFYQH YDLDLKDKPL GEGSFSICRK
 481 CVHKKSNQAF AVKIISKRME ANTQKEITAL KLCEGHPNIV KLHEVFHDQL HTFLVMELLN
 541 GGELFERIKK KKHFSETEAS YIMRKLVSAV SHMHDVGVVH RDLKPENLLF TDENDNLEIK
 601 IIDFGFARLK PPDNQPLKTP CFTLHYAAPE LLNQNGYDES CDLWSLGVIL YTMLSGQVPF
 661 QSHDRSLTCT SAVEIMKKIK KGDFSFEGEA WKNVSQEAKD LIQGLLTVDP NKRLKMSGLR
 721 YNEWLQDGSQ LSSNPLMTPD ILGSSGAAVH TCVKATFHAF NKYKREGFCL QNVDKAPLAK
 781 RRKMKKTSTS TETRSSSSES SHSSSSHSHG KTTPTKTLQP SNPADSNNPE TLFQFSDSVA
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Recombinant MSK1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atctgccacc atggactaca aggacgacga tgacaaggag
 121 gaggagggtg gcagcagcgg cggcgccgcg gggaccagcg cggacggcgg cgacggagga
 181 gagcagctcc tcactgtcaa gcacgagctg cggactgcta atttgacagg acatgctgag
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 361 ttgaaaaagg caacaatcgt tcaaaaggcc aaaaccacag agcatacaag gacagaacga
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 601 cttgccctcg aacatctcca caagttgggg attatatatc gtgatattaa gcttgagaat
 661 attctacttg attctaatgg ccatgtggtg ctgacagatt ttggtctgag taaggagttt
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1201 gccctgcccc agagttctga gaagctgttt cagggctatt cctttgttgc tccttccatc
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1621 ggaggagaac tgtttgagcg cattaagaaa aagaagcact tcagtgagac ggaagccagc
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1681 tacatcatga ggaagcttgt ttcagctgta agccacatgc atgatgttgg agtggtgcac
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2521 tag
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Reviewed and approved by site quality representative.