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Certificate of Analysis

MSK2, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-625, 14-625-K, 14-625M
Parent Lot # D7NN001U

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 MSK2, amino acids 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni2+/NTA-agarose. Purity 59.7% by SDS-PAGE and Coomassie blue staining. MW = 89.9kDa.

Specific Activity (Parent lot# D7NN001U): as provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MAPK2.

Formulation: 1.034mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 0.03% Brij-35, 270mM sucrose. 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.

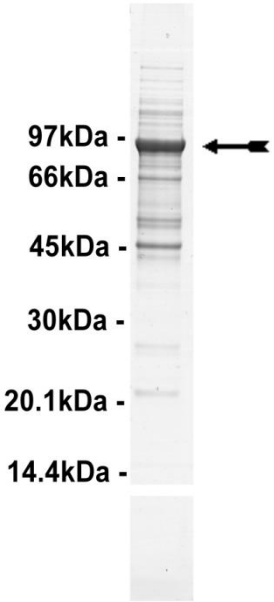
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: etc: Unactive MSK2 was activated using MAPK2 and the increased activity against Crosstide determined. The activation and subsequent assay are described on page two. Results of this assay are shown below.

MAPK2	Unactive MSK2	Mean cpm	Comments
85ng	180ng	41679	Kinase activity
None	360ng	482	Background

MS Tryptic Fingerprint: Confirmed identity as MSK2 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 MSK2, unactive

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Kinase Cascade Assay Protocol

Stock Solutions:

1. **10 x MSK2 activation buffer:** 500mM Tris/HCl pH 7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **5 x MSK2 assay buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
3. **Enzyme Dilution buffer:** Dilute in 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
4. **Magnesium/ATP Cocktail (5 x stock):** 500µM cold ATP, and 50mM magnesium acetate.
5. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500cpm/pmol as required.)
6. **MAPK2, active (Catalogue# 14-173):** Use at a final assay concentration of 0.5µM (0.0339mg/ml). Prepare a 0.339mg/ml stock and add 2.5µl of stock per assay point.
7. **MSK2, unactive:** Use at a final concentration of 2µM (0.1798mg/ml). Prepare a 0.899mg/ml stock and add 5µl of stock per assay point.
8. **Modified Crosstide (GRPRTSSFAEGKK):** Use a final assay concentration of 30µM. Make a 300µM stock. Add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of MSK2 by MAPK2*

1. Add 2.5µl of MSK2 activation buffer to a microcentrifuge tube.
2. Add 2.5µl of **MAPK2, active**.
3. Add **5µl of MSK2, unactive**.
4. Add 10µl of dH_2O.
5. Add 5µl of Magnesium/ATP Cocktail.
6. Incubate for 45 minutes at 30°C.
7. Stop reaction by diluting 2.5–50 fold and placing on ice.

Stage Two: *Phosphorylation of Crosstide by activated MSK2*

1. Add 5µl of MSK2 assay buffer into a microcentrifuge tube.
2. Add 2.5µl of modified Crosstide **(GRPRTSSFAEGKK)**.
3. Add 5µl of **Stage One** reaction product diluted 1–50 fold.
4. Add 2.5µl of dH_2O.
5. Add 10µl of the diluted [γ-^{33}P] ATP.
6. Incubate for 10 minutes at 30°C.
7. Slowly transfer 20µl onto the centre of a 2cm x 2cm **P81** paper.
8. Wash assay squares twice for 5 minutes with 75mM phosphoric acid
9. Wash assay squares once with acetone for 2 minutes.
10. Transfer assay squares to scintillation vials and add 1ml scintillation cocktail.
11. Read in scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all appropriate assay components plus 1µl of 30 % phosphoric acid.

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MSK2 Sequence Information

Protein Human MSK2

Tags *N*-terminal 6His

Native sequence G37 of the recombinant protein is equivalent to G2 of human MSK2

Accession number GenBank AJ010119

Recombinant MSSK2 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SKAYVDGDED DDESCAVELR ITEANLTGHE
 61 EKVSVENFEL LKVLGTGAYG KVFLVRKAGG HDAGKLYAMK VLRKAALVQR AKTQEHTRTE
121 RSVLELVRQA PFLVTLHYAF QTDAKLHLIL DYVSGGEMFT HLYQRQYFKE AEVRVYGGEI
181 VLALEHLHKL GIIYRDLKLE NVLLDSEGHI VLTDFGLSKE FLTEEKERTF SFCGTIEYMA
241 PEIIRSKTGH GKAVDWWSLG ILLFELLTGA SPFTLEGERN TQAEVSRRIL KCSPPFPPRI
301 GPVAQDLLQR LLCKDPKKRL GAGPQGAQEV RNHPFFQGLD WVALAARKIP APFRPQIRSE
361 LDVGNFAEEF TRLEPVYSPP GSPPPGDPRI FQGYSFVAPS ILFDHNNAVM TDGLEAPGAG
421 DRPGRAAVAR SAMMQDSPFF QQYELDLREP ALGQGSFSVC RRCRQRQSGQ EFAVKILSRR
481 LEANTQREVA ALRLCQSHPN VVNLHEVHHD QLHTYLVLEL LRGGELLEHI RKKRHFSESE
541 ASQILRSLVS AVSFMHEEAG VVHRDLKPEN ILYADDTPGA PVKIIDFGFA RLRPQSPGVP
601 MQTPCFTLQY AAPELLAQQG YDESCDLWSL GVILYMMLSG QVPFQGASGQ GGQSQAAEIM
661 CKIREGRFSL DGEAWQGVSE EAKELVRGLL TVDPAKRLKL EGLRGSSWLQ DGSARSSPPL
721 RTPDVLESSG PAVRSGLNAT FMAFNRGKRE GFFLKSVENA PLAKRRKQKL RSATASRRGS
781 PAPANPGRAP VASKGAPRRA NGPLPPS
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Recombinant MSSK2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatccggaat tcaaaggcct acgtcgacgg ggacgaggac
 121 gacgatgaga gctgcgccgt ggagctgcgg atcaccgaag ccaacctgac cgggcacgag
 181 gagaaggtga gcgtggagaa cttcgagctg ctcaaggtgc tgggcacggg agcctacggc
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 301 gtgctgcgca aggcagcgct ggtgcagcgc gccaagacgc aagagcacac gcgcaccgag
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 661 ttcctgacgg aggagaaaga gcggaccttc tccttctgtg gcaccatcga gtacatggcc
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1501 gtggtgaatc tgcacgaggt gcatcacgac cagctgcaca cgtacctggt cctggagctg
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1621 gcaagccaga tcctgcgcag cctcgtgtcg gccgtgagct tcatgcacga ggaggcgggc
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1681 gtggtgcacc gcgacctcaa gccggagaac atcctgtacg ccgacgacac gcccggggcc
1741 ccggtgaaaa tcatcgactt cgggttcgcg cggttgcggc cgcagagtcc cggggtgccc
1801 atgcagacgc cctgcttcac gctgcagtac gctgcccccg agctgctggc gcagcagggc
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2221 ttcatggcat tcaaccgggg caagcgggag ggcttcttcc tgaagagcgt ggagaatgca
2281 cccctggcca agcggcggaa gcagaagctg cggagcgcca ccgcctcccg ccggggctcc
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2401 aacggccccc tgcccccctc ctaa
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Reviewed and approved by site quality representative.