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Standard Operating Procedure

Preparation of active MSK1

Enzyme description:- Active MSK1

Source:- Recombinant

Expression system:- Baculovirus expression vector system (BEVS)/Insect cells

Tag:- His(6)

Purification method:- Ni^{2+}-NTA agarose.

Expression level:- 5 mg/L

Molecular mass:- 95 kDa by SDS-PAGE

Purity:- >90%

Contaminants:- The preparation contains several minor degradation products.

Activation protocol:-

MSK1 (0.2 mg/ml - 2 μM) is activated in 50mM Tris/HCl pH 7.5, 0.1mM EGTA, 0.1 % β-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 2 U/ml active GST-MAPK2/ERK2 at 30°C for 45 min. Following activation, the MSK1 is separated from the GST-MAPK2/ERK2 by incubation on GSH-agarose for 30 min (to remove most of the MAPK2/ERK2 followed by Ni-NTA chromatography (MSK1 binds to the resin by virtue of its His-tag). The re-purified active MSK1 is then eluted from the column and dialysed into enzyme storage buffer, snap frozen in liquid nitrogen prior to storage at –70°C.

Enzyme storage buffer:-

50 mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- -20°C

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CLONE DATA SHEET – human MSK1

Protein	Human MSK1
Accession no	AF074393
Tags	His6 and DYKDDDDK (FLAG) amino-terminal tags
Baculovirus-expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGSATMDYK DDDDKEEEGGSSGGAAGTSADGGDGGEQLLTVKH ELRTANLTGHAEKVGIENFELLKVLGTGAYGKVFLV RKISGHDTGKLYAMKVLKKATIVQKAKTTEHTRTER QVLEHIRQSPFLVTLHYAFQTETKLHLILDYINGGEL FTHLSQRERFTEHEVQIYVGEIVLALEHLHKLGIIYRD IKLENILLDSNGHVVLTDFGLSKEFVADETERAYSFC GTIEYMAPDIVRGGDSGHDKAVDWWSLGVLMYEL LTGASPFTVDGEKNSQAEISRRILKSEPPYPQEMSAL AKDLIQRLLMKDPKKRLGCGPRDADEIKEHLFFQKI NWDDLAAKKVPAPFKPVIRDELDVSNFAEEFTEMD PTYSPAALPQSSEKLFQGYSFVAPSILFKRNAAVIDPL QFHMGVERPGVTNVARSAMMKDSPFYQHYDLDLK DKPLGEGSFSICRKCVHKKSNQAFAVKIISKRMEAN TQKEITALKLCEGHPNIVKLHEVFHDQLHTFLVMEL LNGGELFERIKKKKHFSETEASYIMRKLVSAVSHMH DVGVVHRDLKPENLLFTDENDNLEIKIIDFGFARLKP PDNQPLKTPCFTLHYAAPELLNQNGYDESCDLWSL GVILYTMLSGQVPFQSHDRSLTCTSAVEIMKKIKKGD FSFEGEAWKNVSQEAKDLIQGLLTVDPNKRLKMSG LRYNEWLQDGSQLSSNPLMTPDILGSSGAAVHTCV KATFHAFNKYKREGFCLQNVDKAPLAKRRKMKKT STSTETRSSSSESSHSSSSHSHGKTTPTKTLQPSNPADS NNPETLFQFSDSVA
Native sequence	Residue 40 of the His_6-tagged protein is equivalent to Glu 2 of MSK1. The fusion protein contains an N-terminal His6 tag (residues 5-10) and Flag tag (DYKDDDDK, residues 32-39).
Protease cleavage site	ENLYFQ (rTEV protease) residues 18 – 23 of His_6-tagged protein
Cloning sites	A *Bam*HI site was introduced immediately 5′ to the initiator ATG by PCR. The resulting *Bam*HI/*Kpn*I

fragment was sub-cloned into the *Bam*HI/*Kpn*I sites of pFastBAC HTb.

ORF in baculovirus

AGATCTGCCACCATGGACTACAAGGACGACGATGACAAGGA
GGAGGAGGGTGGCAGCAGCGGCGGCGCCGCGGGGACCAGCG
CGGACGGCGGCGACGGAGGAGAGCAGCTCCTCACTGTCAAG
CACGAGCTGCGGACTGCTAATTTGACAGGACATGCTGAGAA
GGTGGGAATAGAAAATTTTGAGCTCCTGAAGGTCCTAGGAA
CTGGAGCTTATGGAAAAGTATTTCTAGTTCGTAAAATAAGT
GGCCATGATACTGGAAAGCTGTATGCCATGAAAGTTTTGAA
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CAAGGACAGAACGACAAGTCCTGGAACACATTAGGCAGTCG
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CAAACTTCATCTCATTTTAGATTATATAAATGGTGGTGAAC
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TGAAAGAGCATATTCCTTTTGTGGAACTATTGAATACATGG
CACCAGATATTGTCAGAGGGGGAGATTCAGGACATGACAAG
GCAGTTGACTGGTGGAGTTTGGGTGTTCTAATGTATGAATT
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