Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MSK1 [2 - 802]

Enzyme description:- MSK1 [2 - 802]

Clone number:- DU 1849

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and FLAG

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

Expression level:- 5 mg/L

Calculated molecular mass:- 94, 344 daltons

Purity:- >90 %

Activation protocol:-

MSK1 (2 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 2 U/ml GST-SAPK2a [DU 979] at 30 °C for 45 min. Following activation, the MSK1 is re-purified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA, 10 mM magnesium acetate, 0.1 mM sodium vanadate

Substrate:-
Original Crosstide [GRPRTSSFAEG] Final concentration: 30 µM

Specific activity range:- 150 – 300 U/mg

Division of Signal Tranduction Therapy

Clone Data Sheet

MSK1 [2 - 802]

Protein MSK1 [2 – 802]

Clone number DU 1849

Species Human

Accession no AF074393

Tags N-terminal His(6) and Flag (DYKDDDDK)

Baculovirus expressed protein

MSYYHHHHHHDYDIPTTENLYFQGAMGSATMDYKDDDDK**EE**
EGGSSGGAAGTSADGGDGGEQLLTVKHELRTANLTGHAEKV
GIENFELLKVLGTGAYGKVFLVRKISGHDTGKLYAMKVLKK
ATIVQKAKTTEHTRTERQVLEHIRQSPFLVTLHYAFQTETK
LHLILDYINGGELFTHLSQRERFTEHEVQIYVGEIVLALEH
LHKLGIIYRDIKLENILLDSNGHVVLTDFGLSKEFVADETE
RAYSFCGTIEYMAPDIVRGGDSGHDKAVDWWSLGVLMYELL
TGASPFTVDGEKNSQAEISRRILKSEPPYPQEMSALAKDLI
QRLLMKDPKKRLGCGPRDADEIKEHLFFQKINWDDLAAKKV
PAPFKPVIRDELDVSNFAEEFTEMDPTYSPAALPQSSEKLF
QGYSFVAPSILFKRNAAVIDPLQFHMGVERPGVTNVARSAM
MKDSPFYQHYDLDLKDKPLGEGSFSICRKCVHKKSNQAFAV
KIISKRMEANTQKEITALKLCEGHPNIVKLHEVFHDQLHTF
LVMELLNGGELFERIKKKKHFSETEASYIMRKLVSAVSHMH
DVGVVHRDLKPENLLFTDENDNLEIKIIDFGFARLKPPDNQ
PLKTPCFTLHYAAPELLNQNGYDESCDLWSLGVILYTMLSG
QVPFQSHDRSLTCTSAVEIMKKIKKGDFSFEGEAWKNVSQE
AKDLIQGLLTVDPNKRLKMSGLRYNEWLQDGSQLSSNPLMT
PDILGSSGAAVHTCVKATFHAFNKYKREGFCLQNVDKAPLA
KRRKMKKTSTSTETRSSSSESSHSSSSHSHGKTTPTKTLQP
SNPADSNNPETLFQFSDSVA

Native sequence Amino acids E2 – A802 (end) of human MSK1.
Residue E40 of the fusion protein is equivalent to E2 of the native enzyme. The His(6) tag is located at residues 5 – 10 and Flag tag at located at residues 32 - 39

Protease cleavage rTEV (ENLYFQ) residues 18 – 23

Cloning sites *Bam*H1 and *Kpn*1 of pFastBAC HTb

Nucleotide sequence of insert

AGATCTGCCACCATGGACTACAAGGACGACGATGACAAGGA
GGAGGAGGGTGGCAGCAGCGGCGGCGCCGCGGGGACCAGCG
CGGACGGCGGCGACGGAGGAGAGCAGCTCCTCACTGTCAAG
CACGAGCTGCGGACTGCTAATTTGACAGGACATGCTGAGAA
GGTGGGAATAGAAAATTTTGAGCTCCTGAAGGTCCTAGGAA
CTGGAGCTTATGGAAAAGTATTTCTAGTTCGTAAAATAAGT
GGCCATGATACTGGAAAGCTGTATGCCATGAAAGTTTTGAA
AAAGGCAACAATCGTTCAAAAGGCCAAAACCACAGAGCATA
CAAGGACAGAACGACAAGTCCTGGAACACATTAGGCAGTCG
CCATTTTTGGTAACATTACATTATGCTTTCCAGACAGAAAC
CAAACTTCATCTCATTTTAGATTATATAAATGGTGGTGAAC
TTTTTACTCATCTTTCTCAAAGAGAGCGTTTCACAGAGCAT
GAGGTGCAGATTTATGTTGGAGAGATTGTGCTTGCCCTCGA
ACATCTCCACAAGTTGGGGATTATATATCGTGATATTAAGC
TTGAGAATATTCTACTTGATTCTAATGGCCATGTGGTGCTG
ACAGATTTTGGTCTGAGTAAGGAGTTTGTGGCTGATGAAAC
TGAAAGAGCATATTCCTTTTGTGGAACTATTGAATACATGG
CACCAGATATTGTCAGAGGGGGAGATTCAGGACATGACAAG
GCAGTTGACTGGTGGAGTTTGGGTGTTCTAATGTATGAATT
ACTAACTGGAGCATCTCCTTTCACTGTTGATGGAGAAAAAA
ATTCCCAAGCTGAGATATCTAGGAGAATATTAAAAAGTGAG
CCTCCATATCCCCAAGAAATGAGTGCTTTAGCGAAAGACCT
AATTCAGCGTCTTTTGATGAAAGATCCCAAGAAGAGATTGG
GATGTGGTCCACGTGATGCAGATGAAATCAAAGAACATCTC
TTCTTTCAGAAAATAAATTGGGATGATTTAGCCGCCAAAAA
AGTGCCTGCACCATTTAAGCCAGTCATTCGAGATGAATTAG
ATGTGAGTAACTTTGCAGAAGAGTTCACAGAAATGGATCCC
ACTTATTCTCCCGCAGCCCTGCCCCAGAGTTCTGAGAAGCT
GTTTCAGGGCTATTCCTTTGTTGCTCCTTCCATCCTATTCA
AGCGTAATGCAGCTGTCATAGACCCTCTTCAGTTTCACATG
GGAGTTGAACGTCCTGGAGTGACAAATGTTGCCAGGAGTGC
AATGATGAAGGACTCTCCATTCTATCAACACTATGACCTAG
ATTTGAAGGACAAACCCCTGGGAGAAGGTAGTTTTTCAATT
TGTCGAAAGTGTGTGCATAAAAAAAGTAACCAAGCTTTTGC
AGTCAAAATAATCAGCAAAAGGATGGAAGCCAATACTCAAA
AGGAAATAACAGCTCTGAAACTCTGTGAAGGACACCCCAAT
ATTGTGAAGTTGCATGAAGTTTTTCATGATCAGCTTCACAC
GTTTCTAGTGATGGAACTTCTGAATGGAGGAGAACTGTTTG
AGCGCATTAAGAAAAAGAAGCACTTCAGTGAGACGGAAGCC
AGCTACATCATGAGGAAGCTTGTTTCAGCTGTAAGCCACAT
GCATGATGTTGGAGTGGTGCACAGGGATCTGAAACCTGAGA
ATTTATTGTTCACCGATGAAAATGACAATTTGGAAATTAAA
ATAATTGATTTTGGATTTGCACGGCTAAAGCCACCGGATAA

TCAGCCCCTGAAGACTCCATGCTTCACCCTTCATTATGCCG
CCCCAGAGCTCTTGAATCAGAACGGCTACGATGAGTCCTGT
GACCTGTGGAGCTTGGGCGTCATTTTGTACACAATGTTGTC
AGGACAGGTTCCCTTCCAATCTCATGACCGAAGTTTGACGT
GTACCAGCGCGGTGGAAATCATGAAGAAAATTAAAAAGGGA
GATTTCTCCTTTGAAGGAGAAGCCTGGAAGAATGTATCCCA
AGAGGCTAAAGATTTGATCCAAGGACTTCTCACAGTAGATC
CAAACAAAAGGCTTAAAATGTCTGGCTTGAGGTACAATGAA
TGGCTACAAGATGGAAGTCAGCTGTCCTCCAATCCTCTGAT
GACTCCGGATATTCTAGGATCTTCCGGAGCTGCCGTGCATA
CCTGTGTGAAAGCAACCTTCCACGCCTTTAACAAATACAAG
AGAGAGGGGTTTTGCCTTCAGAATGTTGATAAGGCCCCTTT
GGCTAAGAGAAGAAAAATGAAAAAGACTAGCACCAGTACCG
AGACGCGCAGCAGTTCCAGTGAGAGTTCCCATTCTTCTTCC
TCTCATTCTCACGGTAAAACTACACCCACCAAGACACTGCA
GCCCAGCAATCCTGCCGACAGCAATAACCCGGAGACCCTCT
TCCAGTTCTCGGACTCAGTAGCTtaggcatggtaggagtgt
atcagtgatccattgcacctttattccctcagcatatgcct
gaggcgatcttttatgcttttaaaaatgtttcccgttggtc
tcattggaatctgcctcctaatgatttttttcaggaaaac
ctgtttggttatcctcattcaaaagcactggacagagaatg
ttactgtgaatagagcacatattactcttttagcaaccta
gcatgatgccaacaagactattttgaaagagcaaaggttc
ctgtaaatttaattagggctagatttgagctgcttgtaagt
cacaggttttccagatgtctgccaacaagaaatgactcata
ctgtgatgataccttttgctttgccttgtggacaatgtggg
ttttttgaaatttgcacccttcaaacaatgatttatcagaga
aaggggtctgttttcaaaaaagattctgtaatgaattttat
gtgtggcatatacttatttcttgagagaagattttaactta
ttgtttttatttatggttacatatgatgataacctgctat
tattaaactggtacc