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Standard Operating Procedure

Preparation of active JNK1α1 / SAPK1c

Enzyme description:- Active JNK1α1 / SAPK1c

Source:- Recombinant

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

Tag:- His(6)

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

Expression level:- 2-3 mg/L

Molecular mass:- 43 kDa by SDS-PAGE

Purity:- >90%

Contaminants:- No major contaminating proteins as judged by SDS-PAGE.

Activation protocol:-

JNK1α1/ SAPK1c (0.086 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 active GST-MKK4 (13 μg/ml - 200 nM) and GST-MKK7β (15 μg/ml - 200 nM) at 30°C for 40 min. Following activation, the enzyme is separated from the GST-MKK4 and GST-MKK7β by Ni-NTA agarose chromatography (JNK1α1/ SAPK1c binds to the column by virtue of its His-tag). The re-purified active JNK1α1/ SAPK1c is then eluted from the column and dialysed into enzyme storage buffer and fractions are pooled and snap frozen in liquid nitrogen prior to storage at –70°C.

Enzyme storage buffer:-
50 mM Tris/HCl pH 7.5, 270 mM sucrose,150 mM NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET –Human JNK1α1/ SAPK1c

Protein	Human JNK1α1/ SAPK1c
Accession number	L26318
Tags	His(6)
Baculovirus-expressed protein	MHHHHHH**MSRSKRDNNFYSVEIGDSTFTVLK RYQNLKPIGSGAQGIVCAAYDAILERNVAIKK LSRPFQNQTHAKRAYRELVLMKCVNHKNIIGL LNVFTPQKSLEEFQDVYIVMELMDANLCQVIQ MELDHERMSYLLYQMLCGIKHLHSAGIIHRDL KPSNIVVKSDCTLKILDFGLARTAGTSFMMTP YVVTRYYRAPEVILGMGYKENVDLWSVGCIM GEMVCHKILFPGRDYIDQWNKVIEQLGTPCPE FMKKLQPTVRTYVENRPKYAGYSFEKLFPDVL FPADSEHNKLKASQARDLLSKMLVIDASKRIS VDEALQHPYINVWYDPSEAEAPPPKIPDKQLD EREHTIEEWKELIYKEVMDLEERTKNGVIRGQP SPLAQVQQ**
Native sequence	Met8 of the fusion-protein is Met1 of JNK1α1./ SAPK1c There is a His(6)-Tag at residues 2-7.
Protease cleavage site	None
Cloning sites	Nde1/Xhol sites of modified pFastBAC 1.
JNK1α1/SAPK1C NUCLEOTIDE SEQUENCE	ATGCACCATCACCATCACCATATGAGCAGAAGCAAGCGTGACAAC AATTTTTATAGTGTAGAGATTGGAGATTCTACATTCACAGTCCTG AAACGATATCAGAATTTAAAACCTATAGGCTCAGGAGCTCAAGGA ATAGTATGCGCAGCTTATGATGCCATTCTTGAAAGAAATGTTGCA ATCAAGAAGCTAAGCCGACCATTTCAGAATCAGACTCATGCCAAG CGGGCCTACAGAGAGCTAGTTCTTATGAAATGTGTTAATCACAAA AATATAATTGGCCTTTTGAATGTTTTCACACCACAGAAATCCCTA GAAGAATTTCAAGATGTTTACATAGTCATGGACCTCATGGATGCA AATCTTTGCCAAGTGATTCAGATGGAGCTAGATCATGAAAGAATG TCCTACCTTCTCTATCAGATGCTGTGTGGAATCAAGCACCTTCAT TCTGCTGGAATTATTCATCGGGACTTAAAGCCCAGTAATATAGTA GTAAAATCTGATTGCACTTTGAAGATTCTTGACTTCGGTCTGGCC

AGGACTGCAGGAACGAGTTTTATGATGACGCCTTATGTAGTGACT
CGCTACTACAGAGCACCCGAGGTCATCCTTGGCATGGGCTACAAG
GAAAACGTGGATTTATGGTCTGTGGGGTGCATTATGGGAGAAATG
GTTTGCCACAAAATCCTCTTTCCAGGAAGGGACTATATTGATCAG
TGGAATAAAGTTATTGAACAGCTTGGAACACCATGTCCTGAATTC
ATGAAGAAACTGCAACCAACAGTAAGGACTTACGTTGAAAACAGA
CCTAAATATGCTGGATATAGCTTTGAGAAACTCTTCCCTGATGTC
CTTTTCCCAGCTGACTCAGAACACAACAAACTTAAAGCCAGTCAG
GCAAGGGATTTGTTATCCAAAATGCTGGTAATAGATGCATCTAAA
AGGATCTCTGTAGATGAAGCTCTCCAACACCCGTACATCAATGTC
TGGTATGATCCTTCTGAAGCAGAAGCTCCACCACCAAAGATCCCT
GACAAGCAGTTAGATGAAAGGGAACACACAATAGAAGAGTGGAAA
GAATTGATATATAAGGAAGTTATGGACTTGGAGGAGAGAACCAAG
AATGGAGTTATACGGGGGCAGCCCTCTCCTTTAGCACAGGTGCAG
CAGtag