Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active JNK1 alpha 1 [1 - 384]

Enzyme description:- JNK1 alpha 1 [1 – 384]

Clone number:- DU 700

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 2-3 mg/L

Calculated molecular mass:- 45, 153 daltons

Purity:- >90 %

Activation protocol:-

JNK1 alpha 1 (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 MgAc, 0.1 mM ATP with 200 nM activated GST-MKK4 [DU 1788] and 200 nM activated GST-MKK7 beta [DU 703] at 30 °C for 40 min. Following activation, JNK1 is repurified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °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 MgAc

Substrate:-
GST-ATF2 [19 – 96] [DU 1787]: Final concentration: 0.2 mg/ml

Specific activity range:- 30 – 60 U/mg

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Clone Data Sheet

JNK1 alpha 1 [1 - 384]

Protein	JNK1 alpha 1 [1 – 384]
Clone number	DU 700
Species	Human
Accession number	L26318
Tags	N-terminal His(6)
Baculovirus expressed protein	MHHHHHH**MSRSKRDNNFYSVEIGDSTFTVLKRYQNLKPIGSG AQGIVCAAYDAILERNVAIKKLSRPFQNQTHAKRAYRELVLM KCVNHKNIIGLLNVFTPQKSLEEFQDVYIVMELMDANLCQVI QMELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVKS DCTLKILDFGLARTAGTSFMMTPYVVTRYYRAPEVILGMGYK ENVDLWSVGCIMGEMVCHKILFPGRDYIDQWNKVIEQLGTPC PEFMKKLQPTVRTYVENRPKYAGYSFEKLFPDVLFPADSEHN KLKASQARDLLSKMLVIDASKRISVDEALQHPYINVWYDPSE AEAPPPKIPDKQLDEREHTIEEWKELIYKEVMDLEERTKNGV IRGQPSPLAQVQQ**
Native sequence	Amino acids M1 – Q384 (end) of human JNK1 alpha 1. Residue M8 of the fusion protein is equilivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 - 7.
Protease cleavage	None
Cloning sites	*Nde*1 and *Xho*l sites of modified pFastBAC 1

Complete nucleotide sequence

ATGCACCATCACCATCACCATATGAGCAGAAGCAAGCGTGACAACAATT
TTTATAGTGTAGAGATTGGAGATTCTACATTCACAGTCCTGAAACGATA
TCAGAATTTAAAACCTATAGGCTCAGGAGCTCAAGGAATAGTATGCGCA
GCTTATGATGCCATTCTTGAAAGAAATGTTGCAATCAAGAAGCTAAGCC
GACCATTTCAGAATCAGACTCATGCCAAGCGGGCCTACAGAGAGCTAGT
TCTTATGAAATGTGTTAATCACAAAAATATAATTGGCCTTTTGAATGTT
TTCACACCACAGAAATCCCTAGAAGAATTTCAAGATGTTTACATAGTCA
TGGACCTCATGGATGCAAATCTTTGCCAAGTGATTCAGATGGAGCTAGA
TCATGAAAGAATGTCCTACCTTCTCTATCAGATGCTGTGTGGAATCAAG
CACCTTCATTCTGCTGGAATTATTCATCGGGACTTAAAGCCCAGTAATA
TAGTAGTAAAATCTGATTGCACTTTGAAGATTCTTGACTTCGGTCTGGC
CAGGACTGCAGGAACGAGTTTTATGATGACGCCTTATGTAGTGACTCGC
TACTACAGAGCACCCGAGGTCATCCTTGGCATGGGCTACAAGGAAAACG
TGGATTTATGGTCTGTGGGGTGCATTATGGGAGAAATGGTTTGCCACAA
AATCCTCTTTCCAGGAAGGGACTATATTGATCAGTGGAATAAAGTTATT
GAACAGCTTGGAACACCATGTCCTGAATTCATGAAGAAACTGCAACCAA
CAGTAAGGACTTACGTTGAAAACAGACCTAAATATGCTGGATATAGCTT
TGAGAAACTCTTCCCTGATGTCCTTTTCCCAGCTGACTCAGAACACAAC
AAACTTAAAGCCAGTCAGGCAAGGGATTTGTTATCCAAAATGCTGGTAA
TAGATGCATCTAAAAGGATCTCTGTAGATGAAGCTCTCCAACACCCGTA
CATCAATGTCTGGTATGATCCTTCTGAAGCAGAAGCTCCACCACCAAAG
ATCCCTGACAAGCAGTTAGATGAAAGGGAACACACAATAGAAGAGTGGA
AAGAATTGATATATAAGGAAGTTATGGACTTGGAGGAGAGAACCAAGAA
TGGAGTTATACGGGGGCAGCCCTCTCCTTTAGCACAGGTGCAGCAGtag