University of Dundee

Standard Operating Procedure

Preparation of active JNK2 alpha 2 [1 - 424]

Enzyme description:- JNK2 alpha 2 [1 - 424]

Clone number:- DU 699

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 3-5 mg/L

Calculated molecular mass:- 49, 061 daltons

Purity:- >90 %

Activation protocol:-

JNK2 alpha 2 (2μM) 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, JNK2 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:- 60 - 120 U/mg

Clone Data Sheet -JNK2 alpha 2 [1 – 424]

Protein	JNK2 alpha 2 [1 – 424]
Clone number	DU 699
Species	Human
Accession number	L31951
Tags	N-terminal His(6)
Baculovirus expressed protein	MHHHHHH**MSDSKCDSQFYSVQVADSTFTVLKRYQQLKPI GSGAQGIVCAAFDTVLGINVAVKKLSRPFQNQTHAKRAY RELVLLKCVNHKNIISLLNVFTPQKTLEEFQDVYLVMEL MDANLCQVIHMELDHERMSYLLYQMLCGIKHLHSAGIIH RDLKPSNIVVKSDCTLKILDFGLARTACTNFMMTPYVVT RYYRAPEVILGMGYKENVDIWSVGCIMGELVKGCVIFQG TDHIDQWNKVIEQLGTPSAEFMKKLQPTVRNYVENRPKY PGIKFEELFPDWIFPSESERDKIKTSQARDLLSKMLVID PDKRISVDEALRHPYITVWYDPAEAEAPPPQIYDAQLEE REHAIEEWKELIYKEVMDWEERSKNGVVKDQPSDAAVSS NATPSQSSSINDISSMSTEQTLASDTDSSLDASTGPLEG CR**
Native sequence	Amino acids M1 – R424 (end) of human JNK2 alpha 2. 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*1 sites of modified pFastBAC1

Complete nucleotide sequence

ATGCACCATCACCATCACCATATGAGCGACAGTAAATGT
GACAGTCAGTTTTATAGTGTGCAAGTGGCAGACTCAACC
TTCACTGTCCTAAAACGTTACCAGCAGCTGAAACCAATT
GGCTCTGGGGCCCAAGGGATTGTTTGTGCTGCATTTGAT
ACAGTTCTTGGGATAAATGTTGCAGTCAAGAAACTAAGC
CGTCCTTTTCAGAACCAAACTCATGCAAAGAGAGCTTAT
CGTGAACTTGTCCTCTTAAAATGTGTCAATCATAAAAAT
ATAATTAGTTTGTTAAATGTGTTTACACCACAAAAAACT
CTAGAAGAATTTCAAGATGTGTATTTGGTTATGGAATTA
ATGGATGCTAACTTATGTCAGGTTATTCACATGGAGCTG
GATCATGAAAGAATGTCCTACCTTCTTTACCAGATGCTT
TGTGGTATTAAACATCTGCATTCAGCTGGTATAATTCAT
AGAGATTTGAAGCCTAGCAACATTGTTGTGAAATCAGAC
TGCACCCTGAAGATCCTTGACTTTGGCCTGGCCCGGACA
GCGTGCACTAACTTCATGATGACCCCTTACGTGGTGACA
CGGTACTACCGGGCGCCCGAAGTCATCCTGGGTATGGGC
TACAAAGAGAACGTTGATATCTGGTCAGTGGGTTGCATC
ATGGGAGAGCTGGTGAAAGGTTGTGTGATATTCCAAGGC
ACTGACCATATTGATCAGTGGAATAAAGTTATTGAGCAG
CTGGGAACACCATCAGCAGAGTTCATGAAGAAACTTCAG
CCAACTGTGAGGAATTATGTCGAAAACAGACCAAAGTAT
CCTGGAATCAAATTTGAAGAACTCTTTCCAGATTGGATA
TTCCCATCAGAATCTGAGCGAGACAAAATAAAAACAAGT
CAAGCCAGAGATCTGTTATCAAAAATGTTAGTGATTGAT
CCTGACAAGCGGATCTCTGTAGACGAAGCTCTGCGTCAC
CCATACATCACTGTTTGGTATGACCCCGCCGAAGCAGAA
GCCCCACCACCTCAAATTTATGATGCCCAGTTGGAAGAA
AGAGAACATGCAATTGAAGAATGGAAAGAGCTAATTTAC
AAAGAAGTCATGGATTGGGAAGAAAGAAGCAAGAATGGT
GTTGTAAAAGATCAGCCTTCAGATGCAGCAGTAAGTAGC
AACGCCACTCCTTCTCAGTCTTCATCGATCAATGACATT
TCATCCATGTCCACTGAGCAGACGCTGGCCTCAGACACA
GACAGCAGTCTTGATGCCTCGACGGGACCCCTTGAAGGC
TGTCGAtga