

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active c-Jun [1 - 331]

<u>Enzyme description:-</u>	c-Jun [1 - 331]
<u>Clone number:-</u>	DU 968
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E. coli</i>
<u>Tag:-</u>	N-terminal Maltose Binding Protein (MBP) and C-terminal His(6)terminal
<u>Purification method:-</u>	Amylose agarose
<u>Expression level:-</u>	0.5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	82, 080.76 daltons
Average Mass	82, 132.18 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.53
<u>Purity:-</u>	>85 %
<u>Enzyme storage buffer:-</u>	
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.	
<u>Storage temperature:-</u>	-20 °C
<u>Assay:-</u>	Substrate for JNK

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Clone Data Sheet

c-Jun [1 - 331]

<u>Protein</u>	c-Jun [1 - 331]
<u>Clone number</u>	DU 968
<u>Species</u>	Human
<u>Accession no</u>	J04111
<u>Tags</u>	N-terminal MBP and C-terminal His(6)
<u>Baculovirus expressed protein</u>	MKIKTGARILALSALTMMFSASALAKIEEGKLVIWINGDKGYN GLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFW AHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAY PIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFN LQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNAGAKAGLTF LVDLIKMKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTS KVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLEN YLLTDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAQK GEIMPNI PQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSS NNNNNNNNNNLGI<u>IEGR</u>ISEFGSMTAKMETTFYDDALNASFLPSE SGPYGYSNP KILKQSMTLNLADPVGSLKPHLRKNSDLLTSPDV GLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGF AEGFVRALAELHSQNTLPSVTSAAQPVNGAGMVAPAVASVAGGS GSGGFSASLHSEPPVYANLSNFPNGALSSGGGAPSYGAAGLAFP AQPQQQQPPHLPQQMPVQHPRLQALKEEPQTVPEMPGETPPL SPIDMESQERIKAEKRMRNRIAASKCRKRKLERIARLEEKVKT LKAQNSELASTANMLREQVAQLKQKVMNHVNSGCQLMLTQQLQT FHHHHHH
<u>Native sequence</u>	Amino acids M1 – F331 (end) of human c-Jun. Residue M419 of the fusion protein is equivalent to M1 of human c-Jun. The MBP tag is located at residues 1 – 408 and the His(6) tag is located at residues 750 – 755.
<u>Protease cleavage</u>	Factor Xa (<u>IEGR</u>) residues 409 - 412
<u>Cloning sites</u>	<i>Bam</i> H1 sites of pMal2px

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Nucleotide
sequence of insert

GGATCCATGACTGCAAAGATGGAAACGACCTTCTATGGCGATGCCCTCAA
CGCCTCGTTCCTCCCGTCCGAGAGCGGACCTTATGGCTACAGTAACCCCA
AGATCCTGAAACAGAGCATGACCCTGAACCTGGCCGACCCAGTGGGGAGC
CTGAAGCCGCACCTCCGCGCCAAGAACTCGGACCTCCTCACCTCGCCCGA
CGTGGGGCTGCTCAAGCTGGCGTCGCCCAGCTGGAGCGCCTGATAATCC
AGTCCAGCAACGGGACACATCACCACCACGCCGACCCCCACCCAGTTCTTG
TGCCCCAAGAACGTGACAGATGAGCAGGAGGGCTTCGCCGAGGGCTTCGT
GCGCGCCCTGGCCGAACCTGCACAGCCAGAACACGCTGCCCAGCGTCACGT
CGGCGGCGCAGCCGGTCAACGGGGCAGGCATGGTGGCTCCCGCGGTAGCC
TCGGTGGCAGGGGGCAGCGGCAGCGGCGGCTTCAGCGCCAGCCTGCACAG
CGAGCCGCCGGTCTACGCAAACCTCAGCAACTTCAACCCAGGCGCGCTGA
GCAGCGGCGGCGGGGCGCCCTCCTACGGCGCGGCCGGCCTGGCCTTTCCC
GCGCAACCCCAGCAGCAGCAGCAGCCGCCGCACCACCTGCCCCAGCAGAT
GCCCCTGCAGCACCCGCGGCTGCAGGCCCTGAAGGAGGAGCCTCAGACAG
TGCCCGAGATGCCCGGCGAGACACCGCCCCTGTCCCCCATCGACATGGAG
TCCCAGGAGCGGATCAAGGCGGAGAGGAAGCGCATGAGGAACCGCATCGC
TGCCTCCAAGTGCCGAAAAAGGAAGCTGGAGAGAATCGCCCGGCTGGAGG
AAAAAGTGAAACCTTGAAAGCTCAGAACTCGGAGCTGGCGTCCACGGCC
AACATGCTCAGGGAACAGGTGGCACAGCTTAAACAGAAAGTCATGAACCA
CGTTAACAGTGGGTGCCAACTCATGCTAACGCAGCAGTTGCAAACATTTT
ACCATCACCATCACCATtgaggatcc