

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active JNK3 alpha 1 [40 - 422]

<u>Enzyme description:-</u>	JNK3 alpha 1 [40 - 422]
<u>Clone number:-</u>	DU 1511
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His(6)
<u>Purification method:-</u>	GSH Sepharose
<u>Expression level:-</u>	15 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	71, 566.49 daltons
Average Mass	71, 612.87 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.75
<u>Purity:-</u>	>85 %

Activation protocol:-

JNK3 (4 μ M) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 mM MgAc, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 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, JNK3 is repurified by Ni²⁺-NTA agarose chromatography.

Enzyme storage buffer:-

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

Storage temperature:- -70 °C

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

Division of Signal Transduction Therapy

Clone Data Sheet

JNK3 alpha 1 [40 - 422]

<u>Protein</u>	JNK3 alpha 1 [40 - 422]
<u>Clone number</u>	DU 1511
<u>Species</u>	Human
<u>Accession number</u>	NM_002753
<u>Tags</u>	N-terminal GST and C-terminal His(6)
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSSKSKVDNQF YSVEVGDSFTFTVLKRYQNLKPIGSGAQGIVCAAYDAVLDNRNVAIKKLS RPFQONQTHAKRAYRELVLMKCVNHKNIISLLNVFTPOKTLEEFQDVYL VMELMDANLCQVIQMELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKP SNIVVKSDCTLKILDFGLARTAGTSFMMPYVVTRYRRAPEVILGMGY KENVDIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTPCPEFMK KLQPTVRNYVENRPKYAGLTFPKLFPDSLFPADSEHNKLKASQARDLL SKMLVIDPAKRISVDDALQHPYINVWYDPAEVEAPPPQIYDKQLDERE HTIEEWKELIYKEVMNSEKTKNGVVKGQSPSPAQVQQHHHHHH
<u>Native sequence</u>	Amino acids S40 – Q422 (end) of human JNK3 alpha 1. Residue S232 of the fusion protein is equivalent to S40 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) tag is at residues 615 – 620.
<u>Protease cleavage</u>	PreScission site (LEVLFGQP) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 site of pGEX6P-1

Division of Signal Transduction Therapy

Nucleotide sequence of insert

GGATCCAGCAAAAGCAAAGTTGACAACCAGTTCTACAGTGTGGAAGTG
GGAGACTCAACCTTCACAGTTCTCAAGCGCTACCAGAATCTAAAGCCT
ATTGGCTCTGGGGCTCAGGGCATAGTTTGTGCCGCGTATGATGCTGTC
CTTGACAGAAATGTGGCCATTAAGAAGCTCAGCAGACCCTTTCAGAAC
CAAACACATGCCAAGAGAGCGTACCGGGAGCTGGTCCTCATGAAGTGT
GTGAACCATAAAAAACATTATTAGTTTTATTAAATGTCTTCACACCCCAG
AAAACGCTGGAGGAGTTCCAAGATGTTTACTTAGTAATGGAAGTATG
GATGCCAACTTATGTCAAGTGATTCAGATGGAATTAGACCATGAGCGA
ATGTCTTACCTGCTGTACCAAATGTTGTGTGGCATTAAAGCACCTCCAT
TCTGCTGGAATTATTCACAGGGATTTAAAACCAAGTAACATTGTAGTC
AAGTCTGATTGCACATTGAAAATCCTGGACTTTGGACTGGCCAGGACA
GCAGGCACAAGCTTCATGATGACTCCATATGTGGTGACACGTTATTAC
AGAGCCCCCTGAGGTCATCCTGGGGATGGGCTACAAGGAGAACGTGGAT
ATATGGTCTGTGGGATGCATTATGGGAGAAATGGTTCGCCACAAAATC
CTCTTTCCAGGAAGGGACTATATTGACCAGTGGAATAAGGTAATTGAA
CAACTAGGAACACCATGTCCAGAATTCATGAAGAAATTGCAACCCACA
GTAAGAACTATGTGGAGAATCGGCCCAAGTATGCGGGACTCACCTTC
CCCAAACCTCTTCCCAGATTCCCTCTTCCCAGCGGACTCCGAGCACAAT
AAACTCAAAGCCAGCCAAGCCAGGGACTTGTTGTCAAAGATGCTAGTG
ATTGACCCAGCAAAAAGAATATCAGTGGACGACGCCTTACAGCATCCC
TACATCAACGTCTGGTATGACCCAGCCGAAGTGAGGGCGCCTCCACCT
CAGATATATGACAAGCAGTTGGATGAAAGAGAACACACAATTGAAGAA
TGGAAGAAGCTTATCTACAAGGAAGTAATGAATTCAGAAGAAAAGACT
AAAAATGGTGTAGTAAAAGGACAGCCTTCTCCTTCAGCACAGGTGCAG
CAGCATCATCACCATCACCATTtaaggatccgc