

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>	71, 566 daltons
<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:- 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:- 40 - 80 U/mg

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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>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKL TQSM A I RY I ADKHNMLGGCPKERA E I SML E GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGS SKSKVDNQF YSVEVGDSTFTVLKRYQNLKPIGSGAQGIVCAAYDAVLDNRNVAIKKLS RPFQNQTHAKRAYRELVLMKCVNHKNIISLLNVFTPOKTLEEFQDVYL VMELMDANLCQVIQMELDHERMSYLLYQMLCGIKHLHSAGI IHRDLKP SNIVVKSDC TLKILDFGLARTAGT SFMMTPYVVTRYR APEVILGMGY KENVDIWSVGCIMGEMVRHKILFPGRDYIDQWNKVIEQLGTPCPEFMK KLQPTVRNYVENRPKYAGLTFPKLFPDSLFPADSEHNK LKASQARDLL SKMLVIDPAKRISVDDALQHPYINVWYDPAEVEAPPPQIYDKQLDERE HTIEEWKELIYKEVMNSEKTKNGVVKGQPSPSAQVQQHHHHHH</p>
<u>Native sequence</u>	<p>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.</p>
<u>Protease cleavage</u>	PreScission site (<u>LEVLFQGPL</u>) residues 221 – 229
<u>Cloning sites</u>	<i>Bam</i> H1 site of pGEX6P-1

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Nucleotide sequence of insert

GGATCCAGCAAAAGCAAAGTTGACAACCAGTTCTACAGTGTGGAAGTG
GGAGACTCAACCTTCACAGTTCTCAAGCGCTACCAGAATCTAAAGCCT
ATTGGCTCTGGGGCTCAGGGCATAAGTTTGTGCCGCGTATGATGCTGTC
CTTGACAGAAATGTGGCCATTAAGAAGCTCAGCAGACCCTTTCAGAAC
CAAACACATGCCAAGAGAGCGTACCGGGAGCTGGTCCTCATGAAGTGT
GTGAACCATAAAAAACATTATTAGTTTATTAAATGTCTTCACACCCCAG
AAAACGCTGGAGGAGTTCCAAGATGTTTACTTAGTAATGGAAGTGTG
GATGCCAACTTATGTCAAGTGATTCAGATGGAATTAGACCATGAGCGA
ATGTCTTACCTGCTGTACCAAATGTTGTGTGGCATTAAAGCACCTCCAT
TCTGCTGGAATTATTCACAGGGATTTAAAACCAAGTAACATTGTAGTC
AAGTCTGATTGCACATTGAAAATCCTGGACTTTGGACTGGCCAGGACA
GCAGGCACAAGCTTCATGATGACTCCATATGTGGTGACACGTTATTAC
AGAGCCCCCTGAGGTCATCCTGGGGATGGGCTACAAGGAGAACGTGGAT
ATATGGTCTGTGGGATGCATTATGGGAGAAATGGTTCGCCACAAAATC
CTCTTTCCAGGAAGGGACTATATTGACCAGTGGAAATAAGGTAATTGAA
CAACTAGGAACACCATGTCCAGAATTCATGAAGAAATTGCAACCCACA
GTAAGAACTATGTGGAGAATCGGCCCAAGTATGCGGGACTCACCTTC
CCCAAACCTTTCCAGATTCCTCTTTCCAGCGGACTCCGAGCACAAT
AAACTCAAAGCCAGCCAAGCCAGGGACTTGTTGTCAAAGATGCTAGTG
ATTGACCCAGCAAAAAGAATATCAGTGGACGACGCCTTACAGCATCCC
TACATCAACGTCTGGTATGACCCAGCCGAAGTGGAGGCGCCTCCACCT
CAGATATATGACAAGCAGTTGGATGAAAGAGAACACACAATTGAAGAA
TGGAAGAAGCTTATCTACAAGGAAGTAATGAATTCAGAAGAAAAGACT
AAAAATGGTGTAGTAAAAGGACAGCCTTCTCCTTCAGCACAGGTGCAG
CAGCATCATCACCATCACCATtaaggatccgc