Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of JNK1 K55R K56R [1 – 384]

Enzyme description:- JNK1 K55R K56R [1 - 384]

Clone number:- DU 47326

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 71, 063.24 daltons
Average Mass 71, 109.43 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.49

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

JNK1 K55R K56R [1 – 384]

Protein	JNK1 K55R K56R [1 - 384]
Clone number	DU 47326
Species	Human
Accession number	L26318
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSRSKRDNNFYSVE** **IGDSTFTVLKRYQNLKPIGSGAQGIVCAAYDAILERNVAIRRLSRPFQN** **QTHAKRAYRELVLMKCVNHKNIIGLLNVFTPQKSLEEFQDVYIVMELMD** **ANLCQVIQMELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVKS** **DCTLKILDFGLARTAGTSFMMTPYVVTRYYRAPEVILGMGYKENVDLWS** **VGCIMGEMVCHKILFPGRDYIDQWNKVIEQLGTPCPEFMKKLQPTVRTY** **VENRPKYAGYSFEKLFPDVLFPADSEHNKLKASQARDLLSKMLVIDASK** **RISVDEALQHPYINVWYDPSEAEAPPPKIPDKQLDEREHTIEEWKELIY** **KEVMDLEERTKNGVIRGQPSPLAQVQQ**

Native sequence	Amino acids M1 – Q384 (end) of human JNK1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a K55R and a K56R mutation. Residue K55 is equivalent to R286 and K56 is equivalent to R287 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccATGAGCAGAAGCAAGCGTGACAACAATTTTTATAGTGTAGAGATTGGAGATTCTACATTC
ACAGTCCTGAAACGATATCAGAATTTAAAACCTATAGGCTCAGGAGCTCAAGGAATAGTATGCGCA
GCTTATGATGCCATTCTTGAAAGAAATGTTGCAATCAGGAGGCTAAGCCGACCATTTCAGAATCAG
ACTCATGCCAAGCGGGCCTACAGAGAGCTAGTTCTTATGAAATGTGTTAATCACAAAAATATAATT
GGCCTTTTGAATGTTTTCACACCACAGAAATCCCTAGAAGAATTTCAAGATGTTTACATAGTCATG
GAGCTCATGGATGCAAATCTTTGCCAAGTGATTCAGATGGAGCTAGATCATGAAAGAATGTCCTAC
CTTCTCTATCAGATGCTGTGTGGAATCAAGCACCTTCATTCTGCTGGAATTATTCATCGGGACTTA
AAGCCCAGTAATATAGTAGTAAAATCTGATTGCACTTTGAAGATTCTTGACTTCGGTCTGGCCAGG
ACTGCAGGAACGAGTTTTATGATGACGCCTTATGTAGTGACTCGCTACTACAGAGCACCCGAGGTC
ATCCTTGGCATGGGCTACAAGGAAAACGTGGATTTATGGTCCGTGGGGTGCATTATGGGAGAAATG
GTTTGCCACAAAATCCTCTTTCCAGGAAGGGACTATATTGATCAGTGGAATAAAGTTATTGAACAG
CTTGGAACACCATGTCCTGAATTCATGAAGAAACTGCAACCAACAGTAAGGACTTACGTTGAAAAC
AGACCTAAATATGCTGGATATAGCTTTGAGAAACTCTTCCCTGATGTCCTTTTCCCAGCTGACTCA
GAACACAACAAACTTAAAGCCAGTCAGGCAAGGGATTTGTTATCCAAAATGCTGGTAATAGATGCA
TCTAAAAGGATCTCTGTAGATGAAGCTCTCCAACACCCGTACATCAATGTCTGGTATGATCCTTCT
GAAGCAGAAGCTCCACCACCAAAGATCCCTGACAAGCAGTTAGATGAAAGGGAACACACAATAGAA
GAGTGGAAAGAATTGATATATAAGGAAGTTATGGACTTGGAGGAGAGAACCAAGAATGGAGTTATA
CGGGGGCAGCCCTCTCCTTTAGCACAGGTGCAGCAGtaggcggccgc