

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of JIP4 [1 - 1321]

<u>Enzyme description:-</u>	JIP4 [1 - 1321]
<u>Clone number:-</u>	DU 27651
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 146, 114.99 daltons
Average Mass 146, 205.41 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.04

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

JIP4 [1 - 1321]

<u>Protein</u>	JIP4 [1 - 1321]
<u>Clone number</u>	DU 27651
<u>Species</u>	Human
<u>Accession number</u>	NM_001130528.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYI DGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETL KVDFLSKLPKEMLFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLV CFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMELED GVVYQEEPGGSGAVMSERVSGLAGSIYREFERLIGRYDEEVKELMPLVVAVLENLDSV FAQDQEHQVELELLRDDNEQLITQYEREKALRKHAEEKFIEFEDSQEQEKDLQTRVES LESQTRQLELKAKNYADQISRLEEREAEKKEYNALHQHRTHEMIHNYMEHLERTKLHQL SGSDQLESTAHSRIRKERPISLGFPLPAGDGLLTPDAQKGGETPGSEQWKFQELSQPR SHTSLKVSNSPEPQKAVEQDELSDVSOGGSKATTPASTANSDVATIPTDTPLKEENEG FVKVTDAPNKSEISKHIEVQVAQETRVNSTGSAENEEKSEVQAIESTPELMDKDLGSG YKGSSTPTKGIENKAFDRNTESLFEELSSAGSLIGDVDEGADLLGMGREVENLILENT QLLETKNALNIVKNDLIAKVDELTCCKVDLQGELEAVKQAKLKLEKNRELEELRKAR AEAEDARQKAKDDDDSDIPTAQRKRFTRVEMARVLMERNQYKERLMELQEAVRWTEMIR ASRENPAEQEKKRSSIWQFFSRLFSSSSNTTKKPEPPVNLKYNAPTSHVTPSVKKRSST LSQLPGDKSKAFDFLSEETEASLASRREQKREQYRQVKAHVQKEDGRVQAFGWSLPQKY KQVTNGQGENKMNLPVPVYLRPLDEKDTSMKLWCAVGNLSSGGKTRDGGSVVGASVYF KDVAGLDTEGSKQRSASQSSLDKLDQELKEQKELKNQEELSSLVWICTSTHSATKVL I IDAVQPGNILDSTVVCNSHVLCIASVPGARETDYPAGEDLSESGQVDKASLCGSMTSNS SAETDSSLGGITVVGCSAEGVTGAATSPSTNGASPVMKPPMEMEAENSEVDENVPTAEE ATEATEGNAGSAEDTVDISQTG VYTEHVFTDPLGVQIPEDLSPVYQSSNDS DAYKDQIS VLPNEQDLVREEAQKMSSLLPTMWLGAQNGCLYVHSSVAQWRKCLHSIKLKDSILSIVH VKGIVLVALADGTLAIFHRGVDGQWDL SNYHLLDLGRPHHSIRCMTVVHDKVWCGYRNK IYVVQPKAMKIEKSFDAHPRKESQVRQLAWVGDGVVVSIRLDSTLRLYHAHTYQHLQDV DIEPYVSKMLGTGKLGFSFVRITALMVSCNRLWVGTGNGV IISIPLETETNKTSGVPGNR PGSVIRVYGDENS DKVTPGTFIPYCSMAHAQLCFHGHRAVKKFFVAVPGQVISPQSSSS GTDLTGDKAGPSAQEPGSQTPPKSMLVISGGEGYIDFRMGDEGGESELLGEDLPLEPSV TKAERSHLIVWQVMYGN E</p>
<u>Native sequence</u>	Amino acids M1 – E1321 (end) of human JIP4. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGGAGCTGGAGGACGGTGTGGTGTATCAGGAGGAGCCCGGGCGCTCCGGGGCCGTGATGTCGG
AGCGGGTGTCCGGCCTGGCCGGCTCCATCTACCGCGAGTTCGAGCGGCTTATCGGGCGCTATGACGAGGA
GGTGGTCAAAGAGCTGATGCCGCTGGTGGTGGCTGTGCTGGAGAACCTGGACTCGGTGTTCCGCGCAGGAC
CAGGAGCACCAGGTGGAGCTGGAGCTGCTGCGGGACGACAACGAGCAGCTCATCACCCAGTACGAGCGGG
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