

*Division of Signal Transduction Therapy*

**Standard Operating Procedure**

**Preparation of active JAK3 [781 - 1124]**

<b><u>Enzyme description:-</u></b>	JAK3 [781 – 1124]
<b><u>Clone number:-</u></b>	DU 25657
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic          65, 727.55 daltons  
Average Mass          65, 685.00 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                                  5.98

**Purity:-**    >80 %

**Activation protocol:-**                                  Constitutively active

**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 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

**Substrate:-**

KAFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC

Final concentration: 300 uM

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### **Clone Data Sheet**

#### **JAK3 [781 - 1124]**

<b><u>Protein</u></b>	JAK3 [781 - 1124]
<b><u>Clone number</u></b>	DU 25657
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NP_000206.2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Baculovirus expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSR IAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGPLGSMI<b>SSDYELL</b> <b>SDPTPGALAPRDGLWNGAQLYACQDPTIFEERHLKYISQLGKGNFGSV</b> <b>ELCRYDPLGDNTGALVAVKQLQHSQPDQQRDFQREIQILKALHSDFIV</b> <b>KYRGVSYGPRQSLRLVMEYLPSCCLRDFLQRHRARLDASRLLLYSSQ</b> <b>ICKGMEYLGSRRCVHRDLAARNILVESEAHVKIADFG LAKLLPLDKDY</b> <b>YVREPGQSPIFWYAPESLSDNIFSRQSDVWSFGVVLYELFTYCDKSC</b> <b>SPSAEFLRMMGCERDVPALCRLELLEEGQRLPAPPACPAEVHELMKL</b> <b>CWAPSPQDRPSFSALGPQLDMLWSGSRGCETHAFTAHPGKHHSLSFS</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids I781 – S1124 (end) of human JAK3.</p> <p>Residue I233 of the fusion protein is equivalent to I781 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	PreScission site ( <u>LEVLFQGP</u> ) residues 221 – 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> I sites of pFastBac GST 6P1

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**Nucleotide  
sequence of  
insert**

ggatccatgATCTCTTCAGACTATGAGCTCCTCTCAGACCCACACCT  
GGTGCCCTGGCACCTCGTGATGGGCTGTGGAATGGTGCCAGCTCTAT  
GCCTGCCAAGACCCACGATCTTCGAGGAGAGACACCTCAAGTACATC  
TCACAGCTGGGCAAGGGCAACTTTGGCAGCGTGGAGCTGTGCCGCTAT  
GACCCGCTAGGCGACAATACAGGTGCCCTGGTGGCCGTGAAACAGCTG  
CAGCACAGCGGGCCAGACCAGCAGAGGGACTTTCAGCGGGAGATTTCAG  
ATCCTCAAAGCACTGCACAGTGATTTTCATTGTCAAGTATCGTGGTGTC  
AGCTATGGCCCGGGCCGAGAGCCTGCGGCTGGTCATGGAGTACCTG  
CCCAGCGGCTGCTTGC GCGACTTCCTGCAGCGGCACCGCGCGCCTC  
GATGCCAGCCGCTCCTTCTCTATTCTCGCAGATCTGCAAGGGCATG  
GAGTACCTGGGCTCCCGCGCTGCGTGCACCGGACCTGGCCGCCCGA  
AACATCCTCGTGGAGAGCGAGGCACACGTCAAGATCGCTGACTTCGGC  
CTAGCTAAGCTGCTGCCGCTTGACAAAGACTACTACGTGGTCCGCGAG  
CCAGGCCAGAGCCCCATTTCTGGTATGCCCCGAATCCCTCTCGGAC  
AACATCTTCTCTCGCCAGTCAGACGTCTGGAGCTTCGGGGTTCGTCCTG  
TACGAGCTCTTACCTACTGCGACAAAAGCTGCAGCCCCTCGGCCGAG  
TTCCTGCGGATGATGGGATGTGAGCGGGATGTCCCCGCCCTCTGCCGC  
CTCTTGGAAGCTGCTGGAGGAGGGCCAGAGGCTGCCGGCGCCTCCTGCC  
TGCCCTGCTGAGGTTACGAGCTCATGAAGCTGTGCTGGGCCCTAGC  
CCACAGGACCGGCCATCATTTCAGCGCCCTGGGCCCCAGCTGGACATG  
CTGTGGAGCGGAAGCCGGGGGTGTGAGACTCATGCCTTCACTGCTCAC  
CCAGAGGGCAAACACCACTCCCTGTCCTTTTCAtaggcggccgc