Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active JAK2 [809 - 1132]

Enzyme description:- JAK2 [809 – 1132]

Clone number:- DU 19349

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST and C-terminal His(6) tag

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 65, 980.27 daltons
Average Mass 66, 022.90 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.09

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc, 5 mM $MnCl_2$

Substrate:-
PDKtide [KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC]
Final concentration: 100 μM

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Clone Data Sheet

JAK2 [809 - 1132]

Protein	JAK2 [809 – 1132]
Clone Number	DU 19349
Species	Human
Accession number	NP_004963.1
Tags	N-terminal GST and C-terminal His(6)
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFM**FTPDYELLTE** **NDMLPNMRIGALGFSGAFEDRDPTQFEERHLKFLQQLGKGNFGSVEMCR** **YDPLQDNTGEVVAVKKLQHSTEEHLRDFEREIEILKSLQHDNIVKYKGV** **CYSAGRRNLKLIMEYLPYGSLRDYLQKHKERIDHIKLLQYTSQICKGME** **YLGTKRYIHRDLATRNILVENENRVKIGDFGLTKVLPQDKEYYKVKEPG** **ESPIFWYAPESLTESKFSVASDVWSFGVVLYELFTYIEKSKSPPAEFMR** **MIGNDKQGQMIVFHLIELLKNNGRLPRPDGCPDEIYMIMTECWNNNVNQ** **RPSFRDLALRVDQIRDNMAG**HHHHHH
Native sequence	Amino acids F808 – G1132 (end) of human JAK2. Residue F236 of fusion protein is equilivalent to F808 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) tag is located at residues 560 – 565.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Eco*R1 and *Not*1 of pFastBac GST 6P

Nucleotide sequence of insert

gaattcatgTTTACTCCAGATTATGAACTATTAACAGAAAATGACATGT
TACCAAATATGAGGATAGGTGCCCTGGGGTTTTCTGGTGCCTTTGAAGA
CCGGGATCCTACACAGTTTGAAGAGAGACATTTGAAATTTCTACAGCAA
CTTGGCAAGGGTAATTTTGGGAGTGTGGAGATGTGCCGGTATGACCCTC
TACAGGACAACACTGGGGAGGTGGTCGCTGTAAAAAAGCTTCAGCATAG
TACTGAAGAGCACCTAAGAGACTTTGAAAGGGAAATTGAAATCCTGAAA
TCCCTACAGCATGACAACATTGTAAAGTACAAGGGAGTGTGCTACAGTG
CTGGTCGGCGTAATCTAAAATTAATTATGGAATATTTACCATATGGAAG
TTTACGAGACTATCTTCAAAAACATAAAGAACGGATAGATCACATAAAA
CTTCTGCAGTACACATCTCAGATATGCAAGGGTATGGAGTATCTTGGTA
CAAAAAGGTATATCCACAGGGATCTGGCAACGAGAAATATATTGGTGGA
GAACGAGAACAGAGTTAAAATTGGAGATTTTGGGTTAACCAAAGTCTTG
CCACAAGACAAAGAATACTATAAAGTAAAAGAACCTGGTGAAAGTCCCA
TATTCTGGTATGCTCCAGAATCACTGACAGAGAGCAAGTTTTCTGTGGC
CTCAGATGTTTGGAGCTTTGGAGTGGTTCTGTATGAACTTTTCACATAC
ATTGAGAAGAGTAAAAGTCCACCAGCGGAATTTATGCGTATGATTGGCA
ATGACAAACAAGGACAGATGATCGTGTTCCATTTGATAGAACTTTTGAA
GAATAATGGAAGATTACCAAGACCAGATGGATGCCCAGATGAGATCTAT
ATGATCATGACAGAATGCTGGAACAATAATGTAAATCAACGCCCTCCT
TTAGGGATCTAGCTCTTCGAGTGGATCAAATAAGGGATAACATGGCTGG
ACATCACCATCACCATCACtgagcggccgc