Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TYK2 [871 - 1187]

Enzyme description:- TYK2 [871 – 1187]

Clone number:- DU 62932

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 63, 185.82 daltons
Average Mass 63, 226.84 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.95

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, 10 mM DTT, 10 mM Magnesium acetate

Substrate:-
KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC
Final concentration: 300 uM

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Clone Data Sheet

TYK2 [871 - 1187]

Protein	TYK2 [871 - 1187]
Clone number	DU 62932
Species	Human
Accession number	NM_003331.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSM**PHNLADVL** **TVNPDSPASDPTVFHKRYLKKIRDLGEGHFGKVSLYCYDPTNDGTGEM** **VAVKALKADCGPQHRSGWKQEIDILRTLYHEHIIKYKGCCEDQGEKSL** **QLVMEYVPLGSLRDYLPRHSIGLAQLLLFAQQICEGMAYLHSQHYIHR** **DLAARNVLLDNDRLVKIGDFGLAKAVPEGHEYYRVREDGDSPVFWYAP** **ECLKEYKFYYASDVWSFGVTLYELLTHCDSSQSPPTKFLELIGIAQGQ** **MTVLRLTELLERGERLPRPDKCPCEVYHLMKNCWETEASFRPTFENLI** **PILKTVHEKYQGQAPSVFSVC**
Native sequence	Amino acids P871 – C1187 (end) of human TYK2. Residue P233 of the fusion protein is equivalent to P871 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites into pFastBac GST 6P1

Nucleotide sequence of insert

ggatccATGCCCCACAATCTTGCTGACGTCTTGACTGTGAACCCGGAC
TCACCGGCGTCGGACCCTACGGTTTTCCACAAGCGCTATTTGAAAAAG
ATCCGAGATCTGGGCGAGGGTCACTTCGGCAAGGTCAGCTTGTACTGC
TACGATCCGACCAACGACGGCACTGGCGAGATGGTGGCGGTGAAAGCC
CTCAAGGCAGACTGCGGCCCCCAGCACCGCTCGGGCTGGAAGCAGGAG
ATTGACATTCTGCGCACGCTCTACCACGAGCACATCATCAAGTACAAG
GGCTGCTGCGAGGACCAAGGCGAGAAGTCGCTGCAGCTGGTCATGGAG
TACGTGCCCCTGGGCAGCCTCCGAGACTACCTGCCCCGGCACAGCATC
GGGCTGGCCCAGCTGCTGCTCTTCGCCCAGCAGATCTGCGAGGGCATG
GCCTATCTGCACTCGCAGCACTACATCCACCGAGACCTAGCCGCGCGC
AACGTGCTGCTGGACAACGACAGGCTGGTCAAGATCGGGGACTTTGGC
CTAGCCAAGGCCGTGCCCGAAGGCCACGAGTACTACCGCGTGCGCGAG
GATGGGGACAGCCCCGTGTTCTGGTATGCCCCAGAGTGCCTGAAGGAG
TATAAGTTCTACTATGCGTCAGATGTCTGGTCCTTCGGGGTGACCCTG
TATGAGCTGCTGACGCACTGTGACTCCAGCCAGAGCCCCCCCACGAAA
TTCCTTGAGCTCATAGGCATTGCTCAGGGTCAGATGACAGTTCTGAGA
CTCACTGAGTTGCTGGAACGAGGGGAGAGGCTGCCACGGCCCGACAAA
TGTCCCTGTGAGGTCTATCATCTCATGAAGAACTGCTGGGAGACAGAG
GCGTCCTTTCGCCCAACCTTCGAGAACCTCATACCCATTCTGAAGACA
GTCCATGAGAAGTACCAAGGCCAGGCCCCTTCAGTGTTCAGCGTGTGC
tgagcggccgc