MRCPPU REAGENTS and SERVICES

Standard Operating Procedure

Preparation of active IRAK1 [193 - 712]

Enzyme description:- IRAK1 [193 - 712]

Clone number:- DU 39292

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 83, 696.22 daltons
Average Mass 83, 749.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.93

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

Storage temperature:- -70 °C

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

Substrate:-
Myelin Basic Protein Final concentration: 0.33 mg/ml

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Clone Data Sheet

IRAK1 [193 - 712]

Protein IRAK1 [193 - 712]

Clone number DU 39393

Species Human

Accession number P51617

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFQGPLGSPGIPGSTRAA**ARPFPFCWPLCEISRGTHNFSEE**
LKIGEGGFGCVYRAVMRNTVYAVKRLKENADLEWTAVKQSFLTE
VEQLSRFRHPNIVDFAGYCAQNGFYCLVYGFLPNGSLEDRLHCQ
TQACPPLSWPQRLDILLGTARAIQFLHQDSPSLIHGDIKSSNVL
LDERLTPKLGDFGLARFSRFAGSSPSQSSMVARTQTVRGTLAYL
PEEYIKTGRLAVDTDTFSFGVVVLETLAGQRAVKTHGARTKYLK
DLVEEEAEEAGVALRSTQSTLQAGLAADAWAAPIAMQIYKKHLD
PRPGPCPPELGLGLGQLACCCLHRRAKRRPPMTQVYERLEKLQA
VVAGVPGHSEAASCIPPSPQENSYVSSTGRAHSGAAPWQPLAAP
SGASAQAAEQLQRGPNQPVESDESLGGLSAALRSWHLTPSCPLD
PAPLREAGCPQGDTAGESSWGSGPGSRPTAVEGLALGSSASSSS
EPPQIIINPARQKMVQKLALYEDGALDSLQLLSSSSLPGLGLEQ
DRQGPEESDEFQS

Native sequence Amino acids A193 – S712 (end residue) of human IRAK1. Residue A242 of the fusion protein is equivalent to A193 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

gcggccGCGCGCCCCTTTCCGTTTTGCTGGCCCCTCTGTGAGATT
TCCCGGGGCACCCACAACTTCTCGGAGGAGCTCAAGATCGGGGAG
GGTGGCTTTGGGTGCGTGTACCGGGCGGTGATGAGGAACACGGTG
TATGCTGTGAAGAGGCTGAAGGAGAACGCTGACCTGGAGTGGACT
GCAGTGAAGCAGAGCTTCCTGACCGAGGTGGAGCAGCTGTCCAGG
TTTCGTCACCCAAACATTGTGGACTTTGCTGGCTACTGTGCTCAG
AACGGCTTCTACTGCCTGGTGTACGGCTTCCTGCCCAACGGCTCC
CTGGAGGACCGTCTCCACTGCCAGACCCAGGCCTGCCCACCTCTC
TCCTGGCCTCAGCGACTGGACATCCTTCTGGGTACAGCCCGGGCA
ATTCAGTTTCTACATCAGGACAGCCCCAGCCTCATCCATGGAGAC
ATCAAGAGTTCCAACGTCCTTCTGGATGAGAGGCTGACACCCAAG
CTGGGAGACTTTGGCCTGGCCCGGTTCAGCCGCTTTGCCGGGTCC
AGCCCCAGCCAGAGCAGCATGGTGGCCCGGACACAGACAGTGCGG
GGCACCCTGGCCTACCTGCCCGAGGAGTACATCAAGACGGGAAGG
CTGGCTGTGGACACGGACACCTTCAGCTTTGGGGTGGTAGTGCTA
GAGACCTTGGCTGGTCAGAGGGCTGTGAAGACGCACGGTGCCAGG
ACCAAGTATCTGAAAGACCTGGTGGAAGAGGAGGCTGAGGAGGCT
GGAGTGGCTTTGAGAAGCACCCAGAGCACACTGCAAGCAGGTCTG
GCTGCAGATGCCTGGGCTGCTCCCATCGCCATGCAGATCTACAAG
AAGCACCTGGACCCCAGGCCCGGGCCCTGCCCACCTGAGCTGGGC
CTGGGCCTGGGCCAGCTGGCCTGCTGCTGCCTGCACCGCCGGGCC
AAAAGGAGGCCTCCTATGACCCAGGTGTACGAGAGGCTAGAGAAG
CTGCAGGCAGTGGTGGCGGGGGTGCCCGGGCATTCGGAGGCCGCC
AGCTGCATCCCCCCTTCCCCGCAGGAGAACTCCTACGTGTCCAGC
ACTGGCAGAGCCCACAGTGGGGCTGCTCCATGGCAGCCCCTGGCA
GCGCCATCAGGAGCCAGTGCCCAGGCAGCAGAGCAGCTGCAGAGA
GGCCCCAACCAGCCCGTGGAGAGTGACGAGAGCCTAGGCGGCCTC
TCTGCTGCCCTGCGCTCCTGGCACTTGACTCCAAGCTGCCCTCTG
GACCCAGCACCCCTCAGGGAGGCCGGCTGTCCTCAGGGGGACACG
GCAGGAGAATCGAGCTGGGGGAGTGGCCCAGGATCCCGGCCCACA
GCCGTGGAAGGACTGGCCCTTGGCAGCTCTGCATCATCGTCGTCA
GAGCCACCGCAGATTATCATCAACCCTGCCCGACAGAAGATGGTC
CAGAAGCTGGCCCTGTACGAGGATGGGGCCCTGGACAGCCTGCAG
CTGCTGTCGTCCAGCTCCCTCCCAGGCTTGGGCCTGGAACAGGAC
AGGCAGGGGCCCGAAGAAAGTGATGAATTTCAGAGCtgagcggcc
gc