Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of IRAK4 [1 – 460]

Enzyme description:- IRAK4 [1 – 460]

Clone number:- DU 8853

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 78, 303.57 daltons
Average Mass 78, 353.90 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.39

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Standard Filter Binding

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

Myelin Binding Protein Final Concentration: 0.33 mg/ml

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Clone Data Sheet

IRAK4 [1 – 460]

Protein	IRAK4 [1 – 460]
Clone number	DU 8853
Species	Human
Accession number	AAH13316
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MNKPITPST** **YVRCLNVGLIRKLSDFIDPQEGWKKLAVAIKKPSGDDRYNQFHIRRFE** **ALLQTGKSPTSELLFDWGTTNCTVGDLVDLLIQNEFFAPASLLLPDAV** **PKTANTLPSKEAITVQQKQMPFCDKDRTLMTPVQNLEQSYMPPDSSSP** **ENKSLEVSDTRFHSFSFYELKNVTNNFDERPISVGGNKMGEGGFGVVY** **KGYVNNTTVAVKKLAAMVDITTEELKQQFDQEIKVMAKCQHENLVELL** **GFSSDGDDLCLVYVYMPNGSLLDRLSCLDGTPPLSWHMRCKIAQGAAN** **GINFLHENHHIHRDIKSANILLDEAFTAKISDFGLARASEKFAQTVMT** **SRIVGTTAYMAPEALRGEITPKSDIYSFGVVLLEIITGLPAVDEHREP** **QLLLDIKEEIEDEEKTIEDYIDKKMNDADSTSVEAMYSVASQCLHEKK** **NKRPDIKKVQQLLQEMTAS**
Native sequence	Amino acids M1 – S460 (end) of human IRAK4. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

ggatccATGAACAAACCCATAACACCATCAACATATGTGCGCTGCCTC
AATGTTGGACTAATTAGGAAGCTGTCAGATTTTATTGATCCTCAAGAA
GGATGGAAGAAGTTAGCTGTAGCTATTAAAAAACCATCTGGTGATGAT
AGATACAATCAGTTTCACATAAGGAGATTTGAAGCATTACTTCAAACT
GGAAAAAGTCCCACTTCTGAATTACTGTTTGACTGGGGCACCACAAAT
TGCACAGTTGGTGATCTTGTGGATCTTTTGATCCAAAATGAATTTTTT
GCTCCTGCAAGTCTTTTGCTCCCAGATGCTGTTCCCAAAACTGCTAAT
ACACTACCTTCTAAAGAAGCTATAACAGTTCAGCAAAAACAGATGCCT
TTCTGTGACAAAGACAGGACATTGATGACACCTGTGCAGAATCTTGAA
CAAAGCTATATGCCACCTGACTCCTCAAGTCCAGAAAATAAAAGTTTA
GAAGTTAGTGATACACGTTTTCACAGTTTTTCATTTTATGAATTGAAG
AATGTCACAAATAACTTTGATGAACGACCCATTTCTGTTGGTGGTAAT
AAAATGGGAGAGGGAGGATTTGGAGTTGTATATAAAGGCTACGTAAAT
AACACAACTGTGGCAGTGAAGAAGCTTGCAGCAATGGTTGACATTACT
ACTGAAGAACTGAAACAGCAGTTTGATCAAGAAATAAAAGTAATGGCA
AAGTGTCAACATGAAAACTTAGTAGAACTACTTGGTTTCTCAAGTGAT
GGAGATGACCTCTGCTTAGTATATGTTTACATGCCTAATGGTTCATTG
CTAGACAGACTCTCTTGCTTGGATGGTACTCCACCACTTTCTTGGCAC
ATGAGATGCAAGATTGCTCAGGGTGCAGCTAATGGCATCAATTTTCTA
CATGAAAATCATCATATTCATAGAGATATTAAAAGTGCAAATATCTTA
CTGGATGAAGCTTTTACTGCTAAAATATCTGACTTTGGCCTTGCACGG
GCTTCTGAGAAGTTTGCCCAGACAGTCATGACTAGCAGAATTGTGGGA
ACAACAGCTTATATGGCACCAGAAGCTTTGCGTGGAGAAATAACACCC
AAATCTGATATTTACAGCTTTGGTGTGGTTTTACTAGAAATAATAACT
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TCTGTTGCTAGTCAATGTCTGCATGAAAAGAAAAATAAGAGACCAGAC
ATTAAGAAGGTTCAACAGCTGCTGCAAGAGATGACAGCTTCTtaagcg
gccgc