Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of IRAK4 [1 – 460] D329A

Enzyme description:- IRAK4 [1 – 460] D329A

Clone number:- DU 8886

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 78, 259.58 daltons
Average Mass 78, 309.89 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.44

Purity:- >80 %

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

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Clone Data Sheet

IRAK4 [1 – 460] D329A

Protein IRAK4 [1 – 460] D329A

Clone number DU 8886

Species Human

Accession number AAH13316

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MNKPITPST**
YVRCLNVGLIRKLSDFIDPQEGWKKLAVAIKKPSGDDRYNQFHIRRFE
ALLQTGKSPTSELLFDWGTTNCTVGDLVDLLIQNEFFAPASLLLPDAV
PKTANTLPSKEAITVQQKQMPFCDKDRTLMTPVQNLEQSYMPPDSSSP
ENKSLEVSDTRFHSFSFYELKNVTNNFDERPISVGGNKMGEGGFGVVY
KGYVNNTTVAVKKLAAMVDITTEELKQQFDQEIKVMAKCQHENLVELL
GFSSDGDDLCLVYVYMPNGSLLDRLSCLDGTPPLSWHMRCKIAQGAAN
GINFLHENHHIHRDIKSANILLDEAFTAKISAFGLARASEKFAQTVMT
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.

The enzyme has a D329A mutation, which produce a kinase dead enzyme. Residue D168A is equivalent to A560 of the fusion protein

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
CTGGATGAAGCTTTTACTGCTAAAATATCTGCCTTTGGCCTTGCACGG
GCTTCTGAGAAGTTTGCCCAGACAGTCATGACTAGCAGAATTGTGGGA
ACAACAGCTTATATGGCACCAGAAGCTTTGCGTGGAGAAATAACACCC
AAATCTGATATTTACAGCTTTGGTGTGGTTTTACTAGAAATAATAACT
GGACTTCCAGCTGTGGATGAACACCGTGAACCTCAGTTATTGCTAGAT
ATTAAAGAAGAAATTGAAGATGAAGAAAAGACAATTGAAGATTATATT
GATAAAAAGATGAATGATGCTGATTCCACTTCAGTTGAAGCTATGTAC
TCTGTTGCTAGTCAATGTCTGCATGAAAAGAAAAATAAGAGACCAGAC
ATTAAGAAGGTTCAACAGCTGCTGCAAGAGATGACAGCTTCTtaagcg
gccgc