Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Interleukin-1 Receptor-Associated Kinase 4 (IRAK4) [140 - 460]

Enzyme description:- IRAK4 [140 - 460]

Clone number:- DU 15580

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 60, 460.47 daltons
Average Mass 60, 499.44 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.33

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM magnesium acetate

Substrate:-
MBP Final concentration: 0.3 mg/ml

Specific activity range:- To be determined

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Clone Data Sheet

IRAK4 [140 - 460]

Protein	IRAK4 [140 - 460]
Clone number	DU 15580
Species	Human
Accession number	BC013316.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**VSDTRFHSFSFYEL** **KNVTNNFDERPISVGGNKMGEGGFGVVYKGYVNNTTVAVKKLAAMVDIT** **TEELKQQFDQEIKVMAKCQHENLVELLGFSSDGDDLCLVYVYMPNGSLL** **DRLSCLDGTPPLSWHMRCKIAQGAANGINFLHENHHIHRDIKSANILLD** **EAFTAKISDFGLARASEKFAQTVMTSRIVGTTAYMAPEALRGEITPKSD** **IYSFGVVLLEIITGLPAVDEHREPQLLLDIKEEIEDEEKTIEDYIDKKM** **NDADSTSVEAMYSVASQCLHEKKNKRPDIKKVQQLLQEMTAS**
Native sequence	Amino acids V140 – S460 (end) of human IRAK4. Residue V232 of the fusion protein is equivalent to V140 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*1site of pFastBac GST

Nucleotide Sequence of insert

ggatccGTTAGTGATACACGTTTTCACAGTTTTTCATTTTATGAATTGA
AGAATGTCACAAATAACTTTGATGAACGACCCATTTCTGTTGGTGGTAA
TAAAATGGGAGAGGGAGGATTTGGAGTTGTATATAAAGGCTACGTAAAT
AACACAACTGTGGCAGTGAAGAAGCTTGCAGCAATGGTTGACATTACTA
CTGAAGAACTGAAACAGCAGTTTGATCAAGAAATAAAAGTAATGGCAAA
GTGTCAACATGAAAACTTAGTAGAACTACTTGGTTTCTCAAGTGATGGA
GATGACCTCTGCTTAGTATATGTTTACATGCCTAATGGTTCATTGCTAG
ACAGACTCTCTTGCTTGGATGGTACTCCACCACTTTCTTGGCACATGAG
ATGCAAGATTGCTCAGGGTGCAGCTAATGGCATCAATTTTCTACATGAA
AATCATCATATTCATAGAGATATTAAAAGTGCAAATATCTTACTGGATG
AAGCTTTTACTGCTAAAATATCTGACTTTGGCCTTGCACGGGCTTCTGA
GAAGTTTGCCCAGACAGTCATGACTAGCAGAATTGTGGGAACAACAGCT
TATATGGCACCAGAAGCTTTGCGTGGAGAAATAACACCCAAATCTGATA
TTTACAGCTTTGGTGTGGTTTTACTAGAAATAATAACTGGACTTCCAGC
TGTGGATGAACACCGTGAACCTCAGTTATTGCTAGATATTAAAGAAGAA
ATTGAAGATGAAGAAAAGACAATTGAAGATTATATTGATAAAAAGATGA
ATGATGCTGATTCCACTTCAGTTGAAGCTATGTACTCTGTTGCTAGTCA
ATGTCTGCATGAAAAGAAAAATAAGAGACCAGACATTAAGAAGGTTCAA
CAGCTGCTGCAAGAGATGACAGCTTCTtaagcggccgc