

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Interleukin-1 Receptor-Associated Kinase 4 (IRAK4) [160 - 460]

Enzyme description:- IRAK4 [160 - 460]

Clone number:- DU 15580

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

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

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Clone Data Sheet

IRAK4 [160 - 460]

<u>Protein</u>	IRAK4 [160 - 460]
<u>Clone number</u>	DU 15580
<u>Species</u>	Human
<u>Accession number</u>	BC013316.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSVSDTRFHSFSFYEL KNVTNNFDERPISVGGNKMGE GGFVVYKGYVNNTTVAVKKLAAMVDIT TEELKQQFDQEIKVMAKCQHENLVELLGFSSDGD LCLVYVYMPNGSL DRLSCLDGT PPLSWHMRCKIAQGAANGINFLHENHHIHRDIKSANILLD EAF TAKISDFGLARASEKFAQTVMTSRIVGTTAYMAPEALRGEITPKSD IYSFGV LLEIITGLPAVDEHREPQLLLDIKEEIEDEEKTIEDYIDKKM NDADSTSVEAMYSVASQCLHEKKNKRPDIKKVQQLLQEMTAS</p>
<u>Native sequence</u>	<p>Amino acids V160 – S460 (end) of human IRAK4. Residue V232 of the fusion protein is equivalent to V160 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide
Sequence of insert

ggatccGTTAGTGATACACGTTTTTCACAGTTTTTCATTTTATGAATTGA
AGAATGTCACAAATAACTTTGATGAACGACCCATTTCTGTTGGTGGTAA
TAAAATGGGAGAGGGAGGATTTGGAGTTGTATATAAAGGCTACGTAAAT
AACACAACCTGTGGCAGTGAAGAAGCTTGCAGCAATGGTTGACATTACTA
CTGAAGAACTGAAACAGCAGTTTGATCAAGAAATAAAAGTAATGGCAAA
GTGTCAACATGAAAACCTTAGTAGAACTACTTGGTTTTCTCAAGTGATGGA
GATGACCTCTGCTTAGTATATGTTTACATGCCTAATGGTTCATTGCTAG
ACAGACTCTCTTGCTTGGATGGTACTCCACCCTTTCTTGGCACATGAG
ATGCAAGATTGCTCAGGGTGCAGCTAATGGCATCAATTTTCTACATGAA
AATCATCATATTCATAGAGATATTTAAAAGTGCAAATATCTTACTGGATG
AAGCTTTTACTGCTAAAATATCTGACTTTGGCCTTGCACGGGCTTCTGA
GAAGTTTGCCAGACAGTCATGACTAGCAGAATTGTGGGAACAACAGCT
TATATGGCACCAGAAGCTTTGCGTGGAGAAATAACACCCAAATCTGATA
TTTACAGCTTTGGTGTGGTTTTTACTAGAAATAATAACTGGACTTCCAGC
TGTGGATGAACACCGTGAACCTCAGTTATTGCTAGATATTTAAAGAAGAA
ATTGAAGATGAAGAAAAGACAATTGAAGATTATATTGATAAAAAGATGA
ATGATGCTGATTCCACTTCAGTTGAAGCTATGTACTCTGTTGCTAGTCA
ATGTCTGCATGAAAAGAAAAATAAGAGACCAGACATTAAGAAGGTTCAA
CAGCTGCTGCAAGAGATGACAGCTTCTTtaagcggccgc