

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Interleukin-1 Receptor-Associated Kinase 4 (IRAK4) [160 - 460] D329A

Enzyme description:- IRAK4 [160 - 460] D329A

Clone number:- DU 51720

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 60, 416.48 daltons

Average Mass 60, 455.43 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.38

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

IRAK4 [160 - 460] D329A

<u>Protein</u>	IRAK4 [160 - 460] D329A
<u>Clone number</u>	DU 51720
<u>Species</u>	Human
<u>Accession number</u>	AAH13316
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG P LGS VSDTRFHSFSFYEL KNVTNNFDERPISVGGNKMGEGGFVVYKGYVNNTTVAVKKLAAMVDIT TEELKQQFDQEI KVMACQHENLV ELLGFSSDGD LCLVYVYMPNGSLL DRLSCLDGT PPLSWHMRCKIAQGAANGINFLHENHHIHRDIKSANILLD EAF TAKI S A FGLARASEKFAQTVMTSRIVGTTAYMAPEALRGEITPKSD IYSFGVVLLEIITGLPAVDEHREPQLLLDIKEEIEDEEKTIEDYIDKKM 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> <p>The enzyme has a D329A mutation, which produce a kinase dead enzyme. Residue D168A is equivalent to A401 of the fusion protein</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
CTGAAGAACCTGAAACAGCAGTTTGATCAAGAAATAAAAGTAATGGCAAA
GTGTCAACATGAAAACCTTAGTAGAACTACTTGGTTTTCTCAAGTGATGGA
GATGACCTCTGCTTAGTATATGTTTACATGCCTAATGGTTCATTGCTAG
ACAGACTCTCTTGCTTGGATGGTACTCCACCCTTTCTTGGCACATGAG
ATGCAAGATTGCTCAGGGTGCAGCTAATGGCATCAATTTTCTACATGAA
AATCATCATATTCATAGAGATATTAAGTGCAAATATCTTACTGGATG
AAGCTTTTACTGCTAAAATATCTGCCTTTGGCCTTGCACGGGCTTCTGA
GAAGTTTGCCAGACAGTCATGACTAGCAGAATTGTGGGAACAACAGCT
TATATGGCACCAGAAGCTTTGCGTGGAGAAATAACCCCAAATCTGATA
TTTACAGCTTTGGTGTGGTTTTTACTAGAAATAATAACTGGACTTCCAGC
TGTGGATGAACACCGTGAACCTCAGTTATTGCTAGATATTAAGAAGAA
ATTGAAGATGAAGAAAAGACAATTGAAGATTATATTGATAAAAAGATGA
ATGATGCTGATTCCACTTCAGTTGAAGCTATGTACTCTGTTGCTAGTCA
ATGTCTGCATGAAAAGAAAAATAAGAGACCAGACATTAAGAAGGTTCAA
CAGCTGCTGCAAGAGATGACAGCTTCTtaagcggccgc