Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of IkappaB epsilon [2 - 361]

Enzyme description:- IkappaB epsilon [2 – 361]

Clone number:- DU 1034

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	65, 171.05 daltons
Average Mass	65, 212.44 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.10

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

IkappaB epsilon [2 – 361]

Protein	IkappaB epsilon [2 – 361]
Clone number	DU 1034
Species	Human
Accession number	AAM27002.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SEARKGPDEAEESQ** **YDSGIESLRSLRSLPESTSAPASGPSDGSPQPCTHPPGPVKEPQEKEDA** **DGERADSTYGSSSLTYTLSLLGGPEAEDPAPRLPLPHVGALSPQQLEAL** **TYISEDGDTLVHLAVIHEAPAVLLCCLALLPQEVLDIQNNLYQTALHLA** **VHLDQPGAVRALVLKGASRALQDRHGDTALHVACQRQHLACARCLLEGR** **PEPGRGTSHSLDLQLQNWQGLACLHIATLQKNQPLMELLLRNGADIDVQ** **EGTSGKTALHLAVETQERGLVQFLLQAGAQVDARMLNGCTPLHLAAGRG** **LMGISSTLCKAGADSLLRNVEDETPQDLTEESLVLLPFDDLKISGKLLL** **CTD**
Native sequence	Amino acids S2 – D361 (end) of human IkappaB epsilon. Residue S232 of the fusion protein is equivalent to S2 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 *Eco*R1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

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ggatccTCGGAGGCGCGGAAGGGGCCGGACGAGGCGGAGGAGAGCCAGT
ACGACTCTGGCATTGAGTCTCTGCGCTCTCTGCGCTCCCTACCCGAGTC
CACCTCGGCTCCAGCCTCCGGGCCCTCGGACGGCAGCCCCCAGCCCTGC
ACCCATCCTCCGGGACCCGTCAAGGAACCACAGGAGAAGGAAGACGCGG
ATGGGGAGCGGGCTGATTCCACCTATGGCTCCTCCTCGCTCACCTACAC
CCTGTCCTTGCTGGGGGGCCCCGAGGCTGAGGACCCGGCCCCACGCCTG
CCACTCCCCCACGTGGGGGCGCTGAGCCCTCAGCAGCTGGAAGCACTCA
CTTACATCTCCGAGGACGGAGACACGCTGGTCCACCTGGCAGTGATTCA
TGAGGCCCCAGCGGTGCTGCTCTGTTGCCTGGCTTTGCTGCCCCAGGAG
GTCCTGGACATTCAAAATAACCTTTACCAGACAGCACTCCATCTGGCTG
TACATCTGGACCAACCGGGCGCAGTTCGGGCACTGGTGCTGAAGGGGGC
CAGCCGGGCACTACAGGACCGGCATGGTGACACAGCCCTTCATGTGGCC
TGCCAGCGCCAGCACTTGGCCTGTGCCCGCTGCCTGCTGGAAGGGCGGC
CAGAGCCAGGCAGAGGAACATCTCACTCTCTGGACCTCCAGCTGCAAAA
CTGGCAAGGTCTGGCTTGTCTCCACATTGCCACCCTTCAGAAGAACCAA
CCACTCATGGAATTGCTGCTTCGGAATGGAGCTGACATTGATGTGCAGG
AGGGCACCAGTGGTAAGACAGCGCTGCACCTGGCTGTGGAAACCCAAGA
GCGGGGCCTGGTACAGTTCCTGCTCCAGGCTGGTGCCCAGGTAGATGCC
CGCATGCTGAACGGGTGCACACCCCTGCACCTGGCAGCTGGCCGGGGTC
TCATGGGCATCTCATCCACTCTGTGCAAGGCGGGTGCTGACTCCCTGCT
GCGGAATGTGGAGGATGAGACGCCCCAGGACCTGACTGAGGAATCCCTT
GTCCTTTTGCCCTTTGATGACCTGAAGATCTCAGGGAAACTGCTGCTGT
GTACCGACtgagaattc
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