

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of IkappaB beta [2 - 356]

Enzyme description:- IkappaB beta [2 – 356]

Clone number:- DU 1008

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 64, 423.19 daltons

Average Mass 64, 464.06 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.97

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 beta [2 – 356]

<u>Protein</u>	IkappaB beta [2 – 356]
<u>Clone number</u>	DU 1008
<u>Species</u>	Human
<u>Accession number</u>	Q15653.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSAGVACLGKAADADE WCDSGLGSLGPDAAAPGGPGLGAELGPGLSWAPLVFGYVTEGDGTALHL AVIHQHEPFLDFLLGFSAGTEYMDLQNDLGQTALHLAAILGETSTVEKL YAAGAGLCVAERRGHTALHLACRVGAHACARALLQPRRRPREAPDTYL AQGPDRTPDTNHTPVALYPDSLEKEEEESEEDWKLQLEAENYEGHTPL HVAVIHKDVEMVRLLRDAGADLDKPEPTCGRSPLHLAVEAQAADVLELL LRAGANPAARMYGGRTPLGSAMLRPNPILARLLRAHGAPEPEGEDEKSG PCSSSSSDSDSGDEGDEYDDIVVHSSRSQTRL PPTPASKPLPDDPRPV</p>
<u>Native sequence</u>	<p>Amino acids A2 – V356 (end) of human IkappaB beta. Residue A232 of the fusion protein is equivalent to A2 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>Eco</i> R1 sites of pGEX6P-1

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Nucleotide
Sequence Of Insert

ggatccGCTGGGGTCGCGTGCTTGGGAAAAGCTGCCGACGCAGATGAAT
GGTGCACAGCGGCCTGGGCTCCCTGGGTCCGGACGCAGCGGCCCCCGG
AGGACCTGGGTTGGGCGCGGAGTTGGGCCCGGGGCTGTCGTGGGCTCCC
CTCGTCTTCGGCTACGTCACTGAGGATGGGGACACGGCACTGCACTTGG
CTGTGATTCATCAGCATGAACCCCTTCCTGGATTTTCTTCTAGGCTTCTC
GGCCGGCACTGAGTACATGGACCTGCAGAATGACCTAGGCCAGACAGCC
CTGCACCTGGCAGCCATCCTGGGGGAGACATCCACGGTGGAGAAGCTGT
ACGCAGCAGGCGCCGGGCTGTGTGTGGCGGAGCGTAGGGGCCACACGGC
GCTGCACCTGGCCTGCCGTGTGGGGGCACACGCCTGTGCCCGTGCCCTG
CTTCAGCCCCGCCCCGGCGCCCCAGGGAAGCCCCGACACCTACCTCG
CTCAGGGCCCTGACCGTACTCCCGACACCAACCATAACCCCTGTCGCCTT
GTACCCCGATTCCGACTTGGAGAAGGAAGAAGAGGAGAGTGAGGAGGAC
TGGAAGCTGCAGCTGGAGGCTGAAAACCTACGAGGGCCACACCCCCTCC
ACGTGGCCGTTATCCACAAAGATGTGGAGATGGTCCGGCTGCTCCGAGA
TGCTGGAGCTGACCTTGACAAACCGGAGCCACGTGCGGCCGGAGCCCC
CTTCATTTGGCAGTGGAGGCCCAGGCAGCCGATGTGCTGGAGCTTCTCC
TGAGGGCAGGCGGAACCCTGCTGCCCGCATGTACGGTGGCCGCACCCC
ACTCGGCAGTGCCATGCTCCGGCCCAACCCATCCTCGCCCGCCTCCTC
CGTGCACACGGAGCCCCTGAGCCCGAGGGCGAGGACGAGAAATCCGGCC
CCTGCAGCAGCAGTAGCGACAGCGACAGCGGAGACGAGGGCGATGAATA
CGACGACATTGTGGTTCACAGCAGCCGACGCAAACCCGGCTGCCTCCC
ACCCAGCCTCAAACCTCTTCCTGACGACCCCCGCCCCGTGtgagaat
tc