

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of IkappaB alpha [2 - 317]

Protein description:- IkappaB alpha [2 - 317]

Clone number:- DU 1007

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 62, 262.10 daltons

Average Mass 62, 301.90 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.88

Purity:- > 85 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Substrate for IKK beta

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CLONE DATA SHEET

IkappaB alpha [2 - 317]

<u>Protein</u>	IkappaB alpha [2 - 317]
<u>Clone number</u>	DU 1007
<u>Species</u>	Human
<u>Accession number</u>	BC004983
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSFQAAERPQE WAMEGPRDGLKKERLLDDRHDSGLDSMKDEEYEQMVKELQEIRLEPQE VPRGSEPWKQQLTEDGDSFLHLAIHEEKALTMEVIRQVKGDLAFLNF QNNLQOTPLHLAVITNQPEIAEALLGAGCDPELRDFRGNTPLHLACEQ GCLASVGVLTQSC TTPHLHSILKATNYNGHTCLHLASIHGYLGIVELL VSLGADVNAQEP CNGRTALHLAVDLQNPDLVSLLLKCGADVNRVTYQG YSPYQLTWGRPSTRIQQQLGQLTLENLQMLPESEDEESYDTESEFTEF TEDELPYDDCVFGGQRLTL</p>
<u>Native sequence</u>	<p>Amino acids F2 – L317 of human IkappaB alpha. Residue F232 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228

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Cloning sites

*Bam*HI and *Eco*R1 sites of pGEX-6P-1

Nucleotide sequence of insert

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ggatccTTCCAGGCGGCCGAGCGCCCCCAGGAGTGGGCCATGGAGGGC  
CCCCGCGACGGGCTGAAGAAGGAGCGGCTACTGGACGACCGCCACGAC  
AGCGGCCTGGACTCCATGAAAGACGAGGAGTACGAGCAGATGGTCAAG  
GAGCTGCAGGAGATCCGCCTCGAGCCGCAGGAGGTGCCGCGCGGCTCG  
GAGCCCTGGAAGCAGCAGCTCACCGAGGACGGGGACTCGTTCCCTGCAC  
TTGGCCATCATCCATGAAGAAAAGGCACTGACCATGGAAGTGATCCGC  
CAGGTGAAGGGAGACCTGGCCTTCCTCAACTTCCAGAACAACCTGCAG  
CAGACTCCACTCCACTTGGCTGTGATCACCAACCAGCCAGAAATTGCT  
GAGGCACTTCTGGGAGCTGGCTGTGATCCTGAGCTCCGAGACTTTCGA  
GGAAATACCCCCCTACACCTTGCCTGTGAGCAGGGCTGCCTGGCCAGC  
GTGGGAGTCCTGACTCAGTCCTGCACCACCCCGCACCTCCACTCCATC  
CTGAAGGCTACCAACTACAATGGCCACACGTGTCTACACTTAGCCTCT  
ATCCATGGCTACCTGGGCATCGTGGAGCTTTTGGTGTCTTGGGTGCT  
GATGTCAATGCTCAGGAGCCCTGTAATGGCCGGACTGCCCTTCACCTC  
GCAGTGGACCTGCAAAATCCTGACCTGGTGTCACTCCTGTTGAAGTGT  
GGGGCTGATGTCAACAGAGTTACCTACCAGGGCTATTCTCCCTACCAG  
CTCACCTGGGGCCGCCAAGCACCCGGATACAGCAGCAGCTGGGCCAG  
CTGACACTAGAAAACCTTCAGATGCTGCCAGAGAGTGAGGATGAGGAG  
AGCTATGACACAGAGTCAGAGTTCACGGAGTTCACAGAGGACGAGCTG  
CCCTATGATGACTGTGTGTTTGGAGGCCAGCGTCTGACGTTATGAaa  
ttc
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