

Standard Operating Procedure

Preparation of active IKK alpha [1 – 745]

<u>Enzyme description:-</u>	IKK alpha [1 – 745]
<u>Clone number:-</u>	DU 46017
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	112,301.07 daltons
Average Mass	112,373.80 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.20
<u>Purity:-</u>	85 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine	
<u>Storage temperature:-</u>	-70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 10 mM DTT, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
[KKKERLLDDRHDSGLDSMKDEE]	Final concentration: 300 uM

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Clone Data Sheet

IKK alpha [1 – 745]

<u>Protein</u>	IKK alpha [1 – 745]
<u>Clone number</u>	DU 46017
<u>Species</u>	Human
<u>Accession number</u>	NM_001278.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAI PQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL F QGPLGSPGIPGSTRAAAMERPPGLRPGAGGPWEMRERLGTGGFGN VCLYQHRELDLKIAIKSCRLELSTKNRERWCHEIQIMKKLNHANV VKACDVPEELNLIHDVPLLAMEYCSGGDLRKLLNKPENCCGLKE SQILSLLSDIGSGIRYLHENKI IHRDLKPENIVLQDVGGKI IHKI IDLGYAKDVDQGS LCTSFVGT LQYLAPELFENKPYTATVDYWSFG TMVFECIAGYRPFLHHLQPF TWHEKIKKKDPKCIFACEEMS GEVR FSSHLPQPNSLCSLIVEPMENWLQMLNCDPQQRGGPVDLTLKQP RCFVLM DHILNLKIVHILNMTSAKII SFLLPPDES LHSLQSR IER ETGINTGSQELLSETGISLDPRKPASQCVLDGVRGCDSYMVYLF D KSKTVYEGPFASRSLSDCVNYIVQDSKIQLPIIQLRKVWAEAVHY VSGLKEDYSRLFQGR AAML SLLRYNANLTKMKNTLISASQQLKA KLEFFHKSIQLDLERYSEQMTYGISSEKMLKAWKEME EKAIHYAE VGVIGYLEDQIMSLHAEIMELQKSPYGRRQGDLMESLEQRAIDLY KQLKHRPSDHSYSDSTEMVKIIVHTVQSQDRVLKELFGHLSKLLG CKQKI IDLLPKVEVALSNIKEADNTVMFMQGKRQKEIWHLLK IAC TQSSARSLVGSSLEGAVTPQTS AWLPPTSAEHDHSLSCVVTPQDG ETSAQMIEENLNCLGHLSTIIHEANEEQGN SMMNLDWSWLTE
<u>Native sequence</u>	Amino acids M1 – E745 of human IKK alpha. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228

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Cloning sites

Not1 sites of pFB DUAL6P-2.

Nucleotide sequence of insert

gcggccgcgATGGAGCGGCCCCGGGGCTGCGGCCGGGCGCGGGC
GGGCCCTGGGAGATGCGGGAGCGGCTGGGCACCGGCGGCTTCGGG
AACGTCTGTCTGTACCAGCATCGGGAACCTTGATCTCAAAATAGCA
ATTAAGTCTTGTCGCCTAGAGCTAAGTACCAAAAACAGAGAACGA
TGGTGCCATGAAATCCAGATTATGAAGAAGTTGAACCATGCCAAT
GTTGTAAAGGCCTGTGATGTTCTTGAAGAATTGAATATTTTGATT
CATGATGTGCCTCTTCTAGCAATGGAATACTGTTCTGGAGGAGAT
CTCCGAAAGCTGCTCAACAAACCAGAAAATTGTTGTGGACTTAAA
GAAAGCCAGATACTTTCTTTACTAAGTGATATAGGGTCTGGGATT
CGATATTTGCATGAAAACAAAATTATACATCGAGATCTAAAACCT
GAAAACATAGTTCTTCAGGATGTTGGTGGAAGATAATACATAAA
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TGTACATCTTTTGTGGGAACACTGCAGTATCTGGCCCCAGAGCTC
TTTGAGAATAAGCCTTACACAGCCACTGTTGATTATTGGAGCTTT
GGGACCATGGTATTTGAATGTATTGCTGGATATAGGCCTTTTTTG
CATCATCTGCAGCCATTTACCTGGCATGAGAAGATTAAGAAGAAG
GATCCAAAGTGTATATTTGCATGTGAAGAGATGTCAGGAGAAGTT
CGGTTTAGTAGCCATTTACCTCAACCAAATAGCCTTTGTAGTTTA
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GACCCTCAGCAGAGAGGAGGACCTGTTGACCTTACTTTGAAGCAG
CCAAGATGTTTTGTATTAATGGATCACATTTTGAATTTGAAGATA
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GAGGTTGGTGTTCATTGGATACCTGGAGGATCAGATTATGTCTTTG
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CAGGGAGACTTGATGGAATCTCTGGAACAGCGTGCCATTGATCTA
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GCCCTCAGTAATATCAAAGAAGCTGACAATACTGTCTATGTTTCATG
CAGGGGAAAAGGCAGAAAGAAATATGGCATCTCCTTAAAATTGCA
TGTACACAGAGTTCTGCCCCGTCCCTTGTAGGATCCAGTCTAGAA
GGTGCAGTAACCCCTCAGACATCAGCATGGCTGCCCCCGACTTCA

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GCAGAACATGATCATTCTCTGTCATGTGTGGTAACTCCTCAAGAT
GGGGAGACTTCAGCACAAATGATAGAAGAAAATTTGAACTGCCTT
GGCCATTTAAGCACTATTATTCATGAGGCAAATGAGGAACAGGGC
AATAGTATGATGAATCTTGATTGGAGTTGGTTAACAGAAtgagcg
gccgc