

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active IKK epsilon 1 [1 - 716]

<u>Enzyme description:-</u>	IKK epsilon [1 - 716]
<u>Clone number:-</u>	DU 12964
<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>Expression level:-</u>	0.1 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	107, 218.34daltons
Average Mass	107, 286.48daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.9
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<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, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
Myelin Basic Protein	Final concentration: 1 mg/ml
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

IKK epsilon 1 [1 - 716]

Protein IKK epsilon [1 - 716]

Clone number DU 12964

Species Human

Accession number NM_014002

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEELHYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAETSMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMQSTANYLW
HTDDLGGQATASVYKARNKKSSELVAVKVFNTTSYLRPREVQVREFE
VLRKLNHQNIVKLFAVEETGGSRQKVLVMEYCSSGSLLSVLESPENAF
GLPEDEFVLVLRVAVGMNHLRENGIVHRDIKPGNIMRLVGEEGQSIY
KLTDGFAARELDDDEKFSVYGTTEYLHPMYERAVLRKPQKAFGVT
VDLWSIGVTLYHAATGSLPFIPIFGGPRRNKEIMYRITTEKPAIAGA
QRRENGPLEWSYTLPIITCQLSLGLOSQVLPILANILEVEQAKCWGFDQ
FFAETSDILQRVVHVFSLSQAVLHHIYIHAHNTIAIFQEAVHKQTSV
APRHQEYLFEGHLCVLEPSVSAQHIAHTTASSPLTLFSTAIKGLAFR
DPALDVPKFPKVDLQADYNTAKGVLGAGYQALRLARALLDGOELMFR
GLHWVMEVLQATCRRTLEVARTSLLYLSSSLGTERFSSVAGTPEIQEL
KAAAELRSRLRTLAEVLRSRQNTTETQESLSSLNRELVKSRDQVHED
RSIQQIQCCLDKMFYKQFKKSRMRPGLGYNEEQIHKLDKVNFSHLA
KRLQVVFQEECVQKYQASLVTHGKMRVHETRNLRLVGCSSVAACNT
EAQGVQESLSKLEELSHQLLQDRAKGAQASPPPIAPYSPTRKDLLL
HMQELCEGMKLLASDLLDNNRIIERLNRVPAPPDV

Native sequence Amino acids M1 – V716 (end) of human IKKepsilon
Residue M232 of the fusion protein is equivalent to M1 of IKK epsilon.
The GST tag is located at residues 1 - 220 of the fusion protein.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*H1 and *Not*1 of pFastBac GST 6P

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Nucleotide sequence of insert

ggatccATGCAGAGCACAGCCAATTACCTGTGGCACACAGATGACCTG
CTGGGGCAGGGGGCCACTGCCAGTGTGTACAAGGCCCGCAACAAGAAA
TCCGGAGAGCTGGTTGCTGTGAAGGTCTTCAACACTACCAGCTACCTG
CGCCCCGCGAGGTGCAGGTGAGGGAGTTTGAGGTCCTGCGGAAGCTG
AACCACCAGAACATCGTCAAGCTCTTTGCGGTGGAGGAGACGGGCGGA
AGCCGGCAGAAGGTACTGGTGATGGAGTACTGCTCCAGTGGGAGCCTG
CTGAGTGTGCTGGAGAGCCCTGAGAATGCCTTTGGGCTGCCTGAGGAT
GAGTTCCTGGTGGTGTGCTGCGCTGTGTGGTGGCCGGCATGAACCACCTG
CGGGAGAACGGCATTGTGCATCGCGACATCAAGCCGGGAACATCATG
CGCCTCGTAGGGGAGGAGGGGCAGAGCATCTACAAGCTGACAGACTTC
GGCGCTGCCCGGAGCTGGATGATGATGAGAAGTTCGTCTCGGTCTAT
GGGACTGAGGAGTACCTGCATCCCGACATGTATGAGCGGGCGGTGCTT
CGAAAGCCCCAGCAAAAAGCGTTCGGGGTGACTGTGGATCTCTGGAGC
ATTGGAGTGACCTTGTACCATGCAGCCACTGGCAGCCTGCCCTTCATC
CCCTTTGGTGGGCCACGGCGGAACAAGGAGATCATGTACCGGATCACC
ACGGAGAAGCCGGCTGGGGCCATTGCAGGTGCCCAGAGGCGGGAGAAC
GGCCCCCTGGAGTGGAGCTACACCCTCCCCATCACCTGCCAGCTGTCA
CTGGGGCTGCAGAGCCAGCTGGTGGCCATCCTGGCCAACATCCTGGAG
GTGGAGCAGGCCAAGTGCTGGGGCTTCGACCAGTTCCTTTCGCGGAGACC
AGTGACATCCTGCAGCGAGTTGTCTCCATGTCTTCTCCCTGTCCCAG
GCAGTCTGCACCACATCTATATCCATGCCACAACACGATAGCCATT
TTCCAGGAGGCCGTGCACAAGCAGACCAGTGTGGCCCCCGACACCAG
GAGTACCTCTTTGAGGGTCACCTCTGTGTCTTCGAGCCCAGCGTCTCA
GCACAGCACATCGCCACACGACGGCAAGCAGCCCCCTGACCTCTTC
AGCACAGCCATCCCTAAGGGGCTGGCCTTCAGGGACCCTGCTCTGGAC
GTCCCCAAGTTCGTCCCCAAAGTGGACCTGCAGGCGGATTACAACACT
GCCAAGGGCGTGTGGGGCGCCGGCTACCAGGCCCTGCGGCTGGCACGG
GCCCTGCTGGATGGGCAGGAGCTAATGTTTCGGGGGCTGCACTGGGTC
ATGGAGGTGCTCCAGGCCACATGCAGACGGACTCTGGAAGTGGCAAGG
ACATCCCTCCTCTACCTCAGCAGCAGCCTGGGAAGTGAAGGCGGCTGCAGAA
AGCGTGGCTGGAACGCCTGAGATCCAGGAAGTGAAGGCGGCTGCAGAA
CTGAGGTCCAGGCTGCGGACTCTAGCGGAGGTCTCTCCAGATGCTCC
CAAAATATCACGGAGACCCAGGAGAGCCTGAGCAGCCTGAACCGGGAG
CTGGTGAAGAGCCGGGATCAGGTACATGAGGACAGAAGCATCCAGCAG
ATTCAGTGCTGTTTGGACAAGATGAACTTCATCTACAAACAGTTCAAG
AAGTCTAGGATGAGGCCAGGGCTTGGCTACAACGAGGAGCAGATTAC
AAGCTGGATAAGGTGAATTTAGTCAATTTAGCCAAAAGACTCCTGCAG
GTGTTCCAGGAGGAGTGCCTGCAGAAGTATCAAGCGTCTTGTAGTCACA
CACGGCAAGAGGATGAGGGTGGTGCACGAGACCAGGAACCACCTGCGC
CTGGTTGGCTGTTCTGTGGCTGCCTGTAACACAGAAGCCAGGGGGTC
CAGGAGAGTCTCAGCAAGCTCCTGGAAGAGCTATCTCACCAGCTCCTT
CAGGACCGAGCAAAGGGGGCTCAGGCCTCGCCGCTCCCATAGCTCCT
TACCCAGCCCTACACGAAAGGACCTGCTTCTCCACATGCAAGAGCTC
TGCAGGGGATGAAGCTGCTGGCATCTGACCTCCTGGACAACAACCGC
ATCATCGAACGGCTAAATAGAGTCCAGCACCTCCTGATGTCTgagcg
gccgc