

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active IKK epsilon [1 – 716]

<u>Enzyme description:-</u>	IKK epsilon [1 – 745]
<u>Clone number:-</u>	DU 14231
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 107, 218.34 daltons
Average Mass 107, 286.48 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.90

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, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 10 mM DTT, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

Myelin Basic Protein Final concentration: 1 mg/ml

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Clone Data Sheet

IKK epsilon [1 – 716]

<u>Protein</u>	IKK epsilon [1 – 716]
<u>Clone number</u>	DU 14231
<u>Species</u>	Human
<u>Accession number</u>	NM_014002.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSR IAYSKDFETLKVDVFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVL <u>QGPLGSMQSTANYLWHTDDL GQGATASVYKARNKKS GELVAVKV</u> FNTTSYL RPREVQVREFEVL RKLNHQNI VKLFAVEETGGS RQKVL VMEYC SSGSLLSVLESPENAFGLPEDEFV LVR CVVAGMNLREN GIVHRDIKPGNIMRLVGEEGQSIYKLTDFGAARELDDDEK FVSVY GTEEYLHPDMYERAVLRKPOKAFGVTVDLWSIGVTLYHAATGSL PFIPFGGPRRKEIMYRITTEKPA GAIAGAQRRENGPLEWSY TLP ITCQLSLGLQSQ LVPILANILEVEQAKCWGFDQFFAETS DILQRV VVHVFSLSQAVLHHIYIHAHNTIAIFQEA VHKQTSVAPRHQ EYLF EGHLCVLEPSVSAQHIAHTTASSPLTLFSTAI PKGLAFRDPALDV PKFV PKVDLQADYNTAKGVLGAGYQALRLARALLDGOELMFRGLH WMEVLQATCRRTLEVARTSLLYLSSSLGTERFSSVAGTPEIQEL KAAAE LRSRLR TLAEVLSRCSQNI TETQESLSSLNRELVKSRDQV HEDRSIQQIQCCLDKMNFIYKQFKKSRMRPGLGYNEEQIHKLDKV NFSHLAKRLLQVFQEECVQKYQASLVTHGKRM RVVHETRNLRLV GCSVAACNTEAQGVQESLSKLL EELSHQLLODRAKGAQASPPP IA PYPSPTRKDLLLHMQELCEGMKLLASDLLDNNRI IERLNRVPAPP DV</p>
<u>Native sequence</u>	Amino acids M1 – M716 of human IKK alpha. Residue M232 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

*Bam*H1 and *Not*1 sites of pFB DUAL6P-2.

Nucleotide sequence of insert

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ggatccATGCAGAGCACAGCCAATTACCTGTGGCACACAGATGAC
CTGCTGGGGCAGGGGGCCACTGCCAGTGTGTACAAGGCCCGCAAC
AAGAAATCCGGAGAGCTGGTTGCTGTGAAGGTCTTCAACACTACC
AGCTACCTGCGGCCCCGCGAGGTGCAGGTGAGGGAGTTTGAGGTC
CTGCGGAAGCTGAACCACCAGAACATCGTCAAGCTCTTTGCGGTG
GAGGAGACGGGCGGAAGCCGGCAGAAGGTACTGGTGATGGAGTAC
TGCTCCAGTGGGAGCCTGCTGAGTGTGCTGGAGAGCCCTGAGAAT
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GTGGTGGCCGGCATGAACCACCTGCGGGAGAACGGCATTGTGCAT
CGCGACATCAAGCCGGGGAACATCATGCGCCTCGTAGGGGAGGAG
GGGCAGAGCATCTACAAGCTGACAGACTTCGGCGCTGCCCGGGAG
CTGGATGATGATGAGAAGTTCGTCTCGGTCTATGGGACTGAGGAG
TACCTGCATCCCGACATGTATGAGCGGGCGGTGCTTCGAAAGCCC
CAGCAAAAAGCGTTCGGGGTGACTGTGGATCTCTGGAGCATTGGA
GTGACCTTGTACCATGCAGCCACTGGCAGCCTGCCCTTCATCCCC
TTTGGTGGGCCACGGCGGAACAAGGAGATCATGTACCGGATCACC
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AACGGGCCCTGGAGTGGAGCTACACCCTCCCCATCACCTGCCAG
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CACAACACGATAGCCATTTTCCAGGAGGCCGTGCACAAGCAGACC
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AAGGGGGCTCAGGCCTCGCCGCTCCCATAGCTCCTTACCCCAGC
CCTACACGAAAGGACCTGCTTCTCCACATGCAAGAGCTCTGCGAG
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GGGATGAAGCTGCTGGCATCTGACCTCCTGGACAACAACCGCATC
ATCGAACGGCTAAATAGAGTCCCAGCACCTCCTGATGTCtgagcg
gccgc