

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active IKK beta [1 - 736]

<u>Enzyme description:-</u>	IKK beta [1 – 736]
<u>Clone number:-</u>	DU 3167
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 85, 103.24 daltons
Average Mass 85, 157.44 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.12

Purity:- 85 %

Activation protocol:- Constitutively active

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:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

Substrate:-

LDDRHDSGLDSMKDEEY Residues 26 – 42 of IkappaB alpha
Final concentration: 30 μM

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Clone Data Sheet

IKK beta [1 - 736]

<u>Protein</u>	IKK beta [1 - 736]
<u>Clone Number</u>	DU 3167
<u>Species</u>	Human
<u>Accession number</u>	O14920-1
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MHHHHHMSWSPSLTTQTGAWEMKERLGTGGFGNVIRWHNQETGEQIA IKQCRQELS PRNRERWCLEIQIMRRLTHPNVVAARDVPEGMQN LAPNDL PLLAMEYCQGGDLRKYLNQFENCGLREGAILTLLSDIASALRYLHENR IIHRDLKPENIVLQQGEQRLIHKIIDLGYAKELDQGS LCTSFVGTLOYL APELLEQQKYTVTVDYWSFGTLAFECITGFRPFLPNWQPVQWHSKVRQK SEVDIVVSEDLNGTVKFS SLPYPNNLNSVL SERLEKWLQMLMWHPRQ RGTDPTYGPNCGCFKALDDILNLKLVHILNMVTGTIHTYPVTEDESLQSL KARIQODTGIP EEDQELLQEAGLALIPDKPATQCI SDGKLNEGHTLDMD LVFLFDNSKIT YETQISPRPQPE SVSCILQEPKRNLAFFQLRKVWGQVW HSIQTLKEDCNRLQQGQRAAMNLLRNNSCLSKMKNSMASMSQQLKAKL DFFKTSIQIDLEKYSEQTEFGITSDKLLLAWREMEQAVELCGRENEVKL LVERMMALQTDIVDLQRS PMGRKQGGTLD DLEEQARELYRRLREKPRDQ RTEGDSQEMVRLLLQAIQSFEKKVRVIYTQLSKTVVCKQKALELLPKVE EVVSLMNEDEKTVVRLQEKROKELWNLLKIACSKVRGVPVSGSPDSMNAS RLSQPGQLMSQPSTASNSLPEPAKKSEELVAEAHNLCTLLENAIQD TVR EQDQSFTP
<u>Native sequence</u>	Amino acids M1 – P736 of human IKK beta (end residue S756). Residue M8 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.
<u>Protease cleavage</u>	None
<u>Cloning sites</u>	<i>Nde</i> 1 and <i>Not</i> 1 site of pFastBAC modified

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Nucleotide sequence of insert

catATGAGCTGGTCACCTTCCCTGACAACGCAGACATGTGGGGCCTGGG
AAATGAAAGAGCGCCTTGGGACAGGGGATTTGGAAATGTCATCCGATG
GCACAATCAGGAAACAGGTGAGCAGATTGCCATCAAGCAGTGCCGGCAG
GAGCTCAGCCCCCGGAACCGAGAGCGGTGGTGCCTGGAGATCCAGATCA
TGAGAAGGCTGACCCACCCCAATGTGGTGGCTGCCCCGAGATGTCCTGA
GGGGATGCAGAACTTGGCGCCCAATGACCTGCCCCTGCTGGCCATGGAG
TACTGCCAAGGAGGAGATCTCCGGAAGTACCTGAACCAGTTTGAGA
GACTGCTGTGGTCTGCGGGAAGGTGCCATCCTCACCTTGCTGAGTGACATTGC
CTCTGCGCTTAGATACCTTCATGAAAACAGAATCATCCATCGGGATCTA
AAGCCAGAAAACATCGTCCTGCAGCAAGGAGAACAGAGGTTAATACACA
AAATTATTGACCTAGGATATGCCAAGGAGCTGGATCAGGGCAGTCTTTG
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CAGCAGAAGTACACAGTGACCGTCGACTACTGGAGCTTCGGCACCCCTGG
CCTTTGAGTGCATCACGGGCTTCCGGCCCTTCTCCCCAACTGGCAGCC
CGTGCAGTGGCATTCAAAGTGCGGCAGAAGAGTGAGGTGGACATTGTT
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ACCCCAATAATCTTAACAGTGTCTGTCTGAGCGACTGGAGAAGTGGCT
GCAACTGATGCTGATGTGGCACCCCCGACAGAGGGGCACGGATCCCACG
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CCAAGAAGAGTGAAGAACTGGTGGCTGAAGCACATAACCTCTGCACCCCT
GCTAGAAAATGCCATACAGGACACTGTGAGGGAACAAGACCAGAGTTTC
ACGCCctagcgggccgc