

## Standard Operating Procedure

### Preparation of active IKKbeta [1 - 736]

<b><u>Enzyme description:-</u></b>	IKKbeta [1 – 736]
<b><u>Clone number:-</u></b>	DU 3167
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6) tag
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	2 mg/L
<b><u>Calculated molecular mass:-</u></b>	85, 103 daltons
<b><u>Purity:-</u></b>	90 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<b><u>Storage temperature:-</u></b>	-20 °C
<b><u>Assay:-</u></b>	Standard filter binding assay
<b><u>Assay buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc
<b><u>Substrate:-</u></b>	
LDDRHDSGLDSMKDEEY Final concentration: 30 μM	Residues 26 – 42 of IkappaB alpha
<b><u>Specific activity range:-</u></b>	To be determined

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## Clone Data Sheet - IKKbeta [1 - 736]

**Protein** IKKbeta [1 - 736]

**Clone Number** DU 3167

**Species** Human

**Accession number** XM\_032491 [gi: 14738764]  
This record has been removed from the database

**Tags** N-terminal His(6)

**Baculovirus expressed protein**

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MHHHHHMSWSPSLTTQTCGAWEMKERLGTGGFGNVI RWHNQETGEOIA
IKQCRQELSPNRERWCLEIQIMRRLTHPNVVAARDVPEGMONLAPNDL
PLLAMEYCGGDLRKYLNQFENCGLREGAILTLLSDIASALRYLHENR
IIHRDLKPENIVLQOGEQRLIHKIIDLGYAKELDQGSLSLTSFVGTLOYL
APELLEQQKYTVTVDYWSFGTLAFECITGFRPFLPNWQPVQWHSKVRQK
SEVDIVVSEDLNGTVKFSSSLPPYNNLNSVLSERLEKWLQMLMWHPRQ
RGTDPTYGPNCGFKALDDILNLKLVHILNMVTGTIHTYPVTEDESLSL
KARIQODTGIP EEDQELLQEAGLALIPDKPATQCI SDGKLNEGHTLDM
LVFLFDNSKITIYETQISPRPQPEVSVSCILQEPKRNLAFFQLRKVWGQVW
HSIQTLKEDCNRLQOQORAAMNLLRNNSCLSKMKNSMASMSQQLKAKL
DFFKTSIQIDLEKYSEQTEFGITSDKLLAWREMEQAVELCGRENEVKL
LVERMMALQTDIVDLQ RSPMGRKQGGTLDDLEEQARELYRRLREKPRDQ
RTEGDSQEMVRLLLQAIQSFEKKVRVIYTQLSKTVVCKQKALELLPKVE
EVSLSMNEDEKTVVRLQEKROKELWNLLKIACSKVRGVPVSGSPDSMNAS
RLSQPGQLMSQPSTASNSLPEPAKKSEELVAEAHNLCTLLENAIQDTVR
EQDQSFTP
```

**Native sequence** AMINO ACIDS M1 – P736 (END) OF HUMAN IKKBETA.  
Residue M8 of fusion protein is equivalent to M1 of the native enzyme.  
The His(6) tag is located at residues 2 – 7.

**Protease cleavage** None

**Cloning sites** *Nde*1 and *Not*1 site of pFastBAC modified

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**Nucleotide  
sequence of  
insert**

catATGAGCTGGTCACCTTCCCTGACAACGCAGACATGTGGGGCCTGGG  
AAATGAAAGAGCGCCTTGGGACAGGGGGATTTGGAAATGTCATCCGATG  
GCACAATCAGGAAACAGGTGAGCAGATTGCCATCAAGCAGTGCCGGCAG  
GAGCTCAGCCCCCGGAACCGAGAGCGGTGGTGCCTGGAGATCCAGATCA  
TGAGAAGGCTGACCCACCCCAATGTGGTGGCTGCCCCGAGATGTCCCTGA  
GGGGATGCAGAACTTGGCGCCCAATGACCTGCCCCTGCTGGCCATGGAG  
TACTGCCAAGGAGGAGATCTCCGGAAGTACCTGAACCAGTTTGAGA  
GACTGCTGTGGTCTGCGGAAGGTGCCATCCTCACCTTGCTGAGTGACATTGC  
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CAGCAGAAGTACACAGTGACCGTCGACTACTGGAGCTTCGGCACCCCTGG  
CCTTTGAGTGATCACGGGCTTCCGGCCCTTCTCCCAACTGGCAGCC  
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ACCCCAATAATCTTAACAGTGTCTGTCTGAGCGACTGGAGAAGTGGCT  
GCAACTGATGCTGATGTGGCACCCCGACAGAGGGGCACGGATCCCACG  
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CATCAGATAAACTGCTGCTGGCCTGGAGGGAAATGGAGCAGGCTGTGGA  
GCTCTGTGGGCGGAGAACGAAGTGAAACTCCTGGTAGAACGGATGATG  
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CAGCTGATGTCTCAGCCCTCCACGGCCTCCAACAGCTTACCTGAGCCAG  
CCAAGAAGAGTGAAGAACTGGTGGCTGAAGCACATAACCTCTGCACCC  
GCTAGAAAATGCCATACAGGACACTGTGAGGGAACAAGACCAGAGTTTC  
ACGCCctagcggccgc