

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of RelB [2 – 579]

<u>Enzyme description:-</u>	RelB [2 – 579]
<u>Clone number:-</u>	DU 1017
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Cobalt Agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	66, 293.26 daltons
Average Mass	66, 334.99 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.94
<u>Purity:-</u>	>80 %
<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, 1 mM benzamidine, 0.2 mM PMSF	
<u>Storage temperature:-</u>	-70 °C

Division of Signal Transduction Therapy

Clone Data Sheet

RelB [2 – 579]

<u>Protein</u>	RelB [2 – 579]
<u>Clone number</u>	DU 1017
<u>Species</u>	Human
<u>Accession number</u>	NM_006509.3
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	<p>MSYHHHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDLRSGPASGPSVPT GRAMPSRRVARPPAAPELGALGSPDLSSLSLAVSRSTDELEI IDEYIKE NGFGLDGGQPGPGEGLPRLVSRGAASLSTVTLGPVAPPATPPPWGCPLG RLVSPAPGPGPQPHLVITEQPKQRMFRYECEGRSAGSILGESSTEAS KTLPAIELRDCGGLREVEVTACLWWDWPHRVHPSLVGKDCTDGI CRV RLRPHVSPRHSFNNLGIQCVRKKEIEAAIERKIQGLIDPYNAGSLKNHQ EVDMNVVRICFQASYRDQOGQMRMDPVLSEPVYDKKSTNTSELRICRI NKESGPCTGGEELYLLCDKVQKEDI SVVFSRASWEGRADFSQADVHRQI AIVFKTPPYEDLEIVEPVTVNVFLQRLTDGVCSEPLPFTYLPRDHDSYG VDKRRKRGMPDVLGELNSSDPHGIESKRRKKKPAILDHFLPNHGSGPFL PPSALLPDPDFFSGTVSLPGLPEPPGGPDLDDGFAYDPTAPTFTMLDL LPPAPPHASAVVCSGGAGAVVGETPGPEPLTLDYQAPGPGDGGTASLV GSNMFPNHYREAAFGGGLLSPGPEAT</p>
<u>Native sequence</u>	<p>Amino acids L2 – T579 (end) of human RelB. Residue L40 of the fusion protein is equivalent to L2 of the native enzyme. The His(6) tag is located at residues 5 – 10.</p>
<u>Protease cleavage</u>	TEV Protease (ENLYFQG) residues 14 - 21
<u>Cloning sites</u>	<i>Sal</i> I and <i>Kpn</i> I sites of pFastBac HTc

Division of Signal Transduction Therapy

Nucleotide

Sequence of insert

gtcgacCTTCGGTCTGGGCCAGCCTCTGGGCCGTCCTCCCCACTGGCC
GGGCCATGCCGAGTCGCCGCGTCGCCAGACCGCCGGCTGCGCCGGAGCT
GGGGCCTTAGGGTCCCCGACCTCTCCTCACTCTCGCTCGCCGTTTCC
AGGAGCACAGATGAATTGGAGATCATCGACGAGTACATCAAGGAGAACG
GCTTCGGCCTGGACGGGGACAGCCGGGCCCGGGCGAGGGGCTGCCACG
CCTGGTGTCTCGCGGGGCTGCGTCCCTGAGCACGGTCACCCTGGGCCCT
GTGGCGCCCCAGCCACGCCGCCGCTTGGGGCTGCCCCCTGGGCCGAC
TAGTGTCCCCAGCGCCGGGCCCGGGCCCGCAGCCGCACCTGGTCATCAC
GGAGCAGCCCAAGCAGCGCGGCATGCGCTTCCGCTACGAGTGCAGGGC
CGCTCGGCCGGCAGCATCCTTGGGGAGAGCAGCACCGAGGCCAGCAAGA
CGCTGCCCCGCATCGAGCTCCGGGATTGTGGAGGGCTGCGGGAGGTGGA
GGTACTGCCTGCCTGGTGTGGAAGGACTGGCCTCACCGAGTCCACCCC
CACAGCCTCGTGGGGAAAGACTGCACCGACGGCATCTGCAGGGTGCGGC
TCCGGCCTCACGTCAGCCCCGGCACAGTTTTAACAACCTGGGCATCCA
GTGTGTGAGGAAGAAGGAGATTGAGGCTGCCATTGAGCGGAAGATTCAA
CTGGGCATTGACCCTACAACGCTGGGTCCCTGAAGAACCATCAGGAAG
TAGACATGAATGTGGTGAGGATCTGCTTCCAGGCCTCATATCGGGACCA
GCAGGGACAGATGCGCCGGATGGATCCTGTGCTTCCGAGCCCGTCTAT
GACAAGAAATCCACAAACACATCAGAGCTGCGGATTTGCCGAATTAACA
AGGAAAGCGGGCCGTGCACCGGTGGCGAGGAGCTCTACTTGCTCTGCGA
CAAGGTGCAGAAAGAGGACATATCAGTGGTGTTCAGCAGGGCCTCCTGG
GAAGGTGCGGGCTGACTTCTCCCAGGCCGACGTGCACCGCCAGATTGCCA
TTGTGTTCAAGACGCCGCCCTACGAGGACCTGGAGATTGTCGAGCCCGT
GACAGTCAACGTCTTCTGTCAGCGGCTCACCGATGGGGTCTGCAGCGAG
CCATTGCCTTTCACGTACCTGCCTCGCGACCATGACAGCTACGGCGTGG
ACAAGAAGcgGAAACGGGGGATGCCCGACGTCTTGGGGAGCTGAACAG
CTCTGACCCCCATGGCATCGAGAGCAAACGGCGGAAGAAAAAGCCGGCC
ATCCTGGACCACTTCTTGCCCAACCACGGCTCAGGCCCGTTCTTCCC
CGTCAGCCCTGCTGCCAGACCCTGACTTCTTCTGTCACCGTGTCCCT
GCCCCGCCTGGAGCCCCCTGGCGGGCCTGACCTCCTGGACGATGGCTTT
GCCTACGACCCTACGGCCCCACACTCTTACCATGCTGGACCTGCTGC
CCCCGGCACCGCCACACGCTAGCGCTGTTGTGTGCAGCGGAGGTGCCGG
GGCCGTGGTTGGGGAGACCCCCGGCCCTGAACCACTGACACTGGACTCG
TACCAGGCCCGGGCCCCGGGGATGGAGGCACCGCCAGCCTTGTGGGCA
GCAACATGTTCCCCAATCATTACCGCGAGGCGGCCTTTGGGGGCGGCCT
CCTATCCCCGGGGCCTGAAGCCACGtagggtacc