Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of RelA [2 – 551]

Enzyme description:- RelA [2 – 551]

Clone number:- DU 1005

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-
Monoisotopic 63, 419.75 daltons
Average Mass 63, 459.61 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.58

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

RelA [2 – 551]

Protein	RelA [2 – 551]
Clone number	DU 1005
Species	Human
Accession number	Q04206.2
Tags	N-terminal His6
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**DELFPLIFPAEPAQASGPYVE IIEQPKQRGMRFRYKCEGRSAGSIPGERSTDTTKTHPTIKINGYTGPGT VRISLVTKDPPHRPHPHELVGKDCRDGFYEAELCPDRCIHSFQNLGIQC VKKRDLEQAISQRIQTNNNPFQVPIEEQRGDYDLNAVRLCFQVTVRDPS GRPLRLPPVLSHPIFDNRAPNTAELKICRVNRNSGSCLGGDEIFLLCDK VQKEDIEVYFTGPGWEARGSFSQADVHRQVAIVFRTPPYADPSLQAPVR VSMQLRRPSDRELSEPMEFQYLPDTDDRHRIEEKRKRTYETFKSIMKKS PFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPT MVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLA PGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVF TDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRPPD PAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLSQISS**

Native sequence	Amino acids D2 – S551 (end) of human RelA. Residue D29 of the fusion protein is equivalent to D2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	TEV Protease (ENLYFQG) residues 14 - 21
Cloning sites	*Bam*H1 and *Kpn*1 sites of pFastBac HTb

Nucleotide Sequence of insert

ggatccgACGAACTGTTCCCCCTCATCTTCCCGGCAGAGCCAGCCCAG
GCCTCTGGCCCCTATGTGGAGATCATTGAGCAGCCCAAGCAGCGGGGCA
TGCGCTTCCGCTACAAGTGCGAGGGGCGCTCCGCGGGCAGCATCCCAGG
CGAGAGGAGCACAGATACCACCAAGACCCACCCCACCATCAAGATCAAT
GGCTACACAGGACCAGGGACAGTGCGCATCTCCCTGGTCACCAAGGACC
CTCCTCACCGGCCTCACCCCCACGAGCTTGTAGGAAAGGACTGCCGGGA
TGGCTTCTATGAGGCTGAGCTCTGCCCGGACCGCTGCATCCACAGTTTC
CAGAACCTGGGAATCCAGTGTGTGAAGAAGCGGGACCTGGAGCAGGCTA
TCAGTCAGCGCATCCAGACCAACAACAACCCCTTCCAAGTTCCTATAGA
AGAGCAGCGTGGGGACTACGACCTGAATGCTGTGCGGCTCTGCTTCCAG
GTGACAGTGCGGGACCCATCAGGCAGGCCCCTCCGCCTGCCGCCTGTCC
TTTCTCATCCCATCTTTGACAATCGTGCCCCCAACACTGCCGAGCTCAA
GATCTGCCGAGTGAACCGAAACTCTGGCAGCTGCCTCGGTGGGGATGAG
ATCTTCCTACTGTGTGACAAGGTGCAGAAAGAGGACATTGAGGTGTATT
TCACGGGACCAGGCTGGGAGGCCCGAGGCTCCTTTTCGCAAGCTGATGT
GCACCGACAAGTGGCCATTGTGTTCCGGACCCCTCCCTACGCAGACCCC
AGCCTGCAGGCTCCTGTGCGTGTCTCCATGCAGCTGCGGCGGCCTTCCG
ACCGGGAGCTCAGTGAGCCCATGGAATTCCAGTACCTGCCAGATACAGA
CGATCGTCACCGGATTGAGGAGAAACGTAAAAGGACATATGAGACCTTC
AAGAGCATCATGAAGAAGAGTCCTTTCAGCGGACCCACCGACCCCCGGC
CTCCACCTCGACGCATTGCTGTGCCTTCCCGCAGCTCAGCTTCTGTCCC
CAAGCCAGCACCCCAGCCCTATCCCTTTACGTCATCCCTGAGCACCATC
AACTATGATGAGTTTCCCACCATGGTGTTTCCTTCTGGGCAGATCAGCC
AGGCCTCGGCCTTGGCCCCGGCCCCTCCCCAAGTCCTGCCCCAGGCTCC
AGCCCCTGCCCCTGCTCCAGCCATGGTATCAGCTCTGGCCCAGGCCCCA
GCCCCTGTCCCAGTCCTAGCCCCAGGCCCTCCTCAGGCTGTGGCCCCAC
CTGCCCCCAAGCCCACCCAGGCTGGGGAAGGAACGCTGTCAGAGGCCCT
GCTGCAGCTGCAGTTTGATGATGAAGACCTGGGGGCCTTGCTTGGCAAC
AGCACAGATCCAGCTGTGTTCACAGACCTGGCATCCGTCGACAACTCCG
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AGAGCCCATGCTGATGGAGTACCCTGAGGCTATAACTCGCCTAGTGACA
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GGCTCCCCAATGGCCTCCTTTCAGGAGATGAAGACTTCTCCTCCATTGC
GGACATGGACTTCTCAGCCCTGCTGAGTCAGATCAGCTCCtaaggtacc