

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RICK [2 - 540]

<u>Enzyme description:-</u>	RICK [2 - 540]
<u>Clone number:-</u>	DU 1182
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	65, 875.99 daltons
Average Mass	65, 834.32 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.55
<u>Purity:-</u>	>85 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
Myelin Basic Protein Final concentration: 0.3 mg/ml	
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

RICK [2 - 540]

<u>Protein</u>	RICK [2 - 540]
<u>Clone number</u>	DU 1182
<u>Species</u>	Human
<u>Accession number</u>	NP_003812
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGSGIQRPSTSTSSLVAAANGEAIC SALPTIPYHKLADLRYLSRGASGTVSSARHADWRVQVAVKHLHIHTPLL DSERKDVLR AEILHKARFSYILPILGICNEPEFLGIVTEYMPNGSLNE LLHRKTEYPDVAWPLRFRI LHEIALGVNYLHNMTPELLHDLKTQNILL DNEFHVKIADFGLSKWRMMSLSQSRSSKSAPEGGTIIYMPPENYE PGQK SRASIKHDIYSYAVITWEVLSRKQPFEDVTNPLQIMYSVSQGHRPVINE ESLPYDIPHRARMISLIESGWAQNPDERPSFLKCLIELEPVLRTFEEIT FLEAVIQLKKTQLQSVSSAIHLCDK KKMELSLNIPVNHGPQEESCGSSQ LHENSGSPETSRSRLPAPQDNDFLSRKAQDCYFMKLHHC PGNHSWDSTIS GSQRAAFCDHKTTPCSSAIINPLSTAGNSERLQPGIAQQW IQSKREDIV NQMT EACLNQSLDALLSRDLIMKEDYELVSTKPTRTSKVRQLLDTTDIQ GEEFAKVIVQKLKDNKQMGLOPYPEILVVSRSPSLNLLQNKSM
<u>Native sequence</u>	Amino acids N2 – M540 (end) of human RICK. Residue N44 of the fusion protein is equivalent to N2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Not1</i> in pFastBAC HTb

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Nucleotide insert sequence

gcggccgcccAACGGGGAGGCCATCTGCAGCGCCCTGCCCACCATTCCCT
ACCACAAACTCGCCGACCTGCGCTACCTGAGCCGCGGCGCCTCTGGCAC
TGTGTCGTCCGCCCCGCCACGCAGACTGGCGCGTCCAGGTGGCCGTGAAG
CACCTGCACATCCACACTCCGCTGCTCGACAGTGAAAGAAAGGATGTCT
TAAGAGAAGCTGAAATTTTACACAAAGCTAGATTTAGTTACATTCTTCC
AATTTTGGGAATTGCAATGAGCCTGAATTTTGGGAATAGTTACTGAA
TACATGCCAAATGGATCATTAATGAACCTCCTACATAGGAAAACCTGAAT
ATCCTGATGTTGCTTGGCCATTGAGATTTTCGCATCCTGCATGAAATTGC
CCTTGGTGTAAATTACCTGCACAATATGACTCCTCCTTTACTTCATCAT
GACTTGAAGACTCAGAATATCTTATTGGACAATGAATTTTCATGTTAAGA
TTGCAGATTTTGGTTTATCAAAGTGGCGCATGATGTCCCTCTCACAGTC
ACGAAGTAGCAAATCTGCACCAGAAGGAGGGACAATTATCTATATGCCA
CCTGAAAACCTATGAACCTGGACAAAAATCAAGGGCCAGTATCAAGCACG
ATATATATAGCTATGCAGTTATCACATGGGAAGTGTTATCCAGAAAACA
GCCTTTTGAAGATGTCACCAATCCTTTGCAGATAATGTATAGTGTGTCA
CAAGGACATCGACCTGTTATTAATGAAGAAAGTTTGCCATATGATATAC
CTCACCGAGCACGTATGATCTCTCTAATAGAAAGTGGATGGGCACAAAA
TCCAGATGAAAGACCATCTTTCTTAAAATGTTTAATAGAACCTGAACCA
GTTTTGAGAACATTTGAAGAGATAACTTTTCTTGAAGCTGTTATTCAGC
TAAAGAAAACAAAGTTACAGAGTGTTTCAAGTGCCATTCACCTATGTGA
CAAGAAGAAAATGGAATTATCTCTGAACATACCTGTAAATCATGGTCCA
CAAGAGGAATCATGTGGATCCTCTCAGCTCCATGAAAATAGTGGTTCTC
CTGAAACTTCAAGGTCCCTGCCAGCTCCTCAAGACAATGATTTTTTTATC
TAGAAAAGCTCAAGACTGTTATTTTATGAAGCTGCATCACTGTCCTGGA
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GTGATCACAAGACCACTCCATGCTCTTCAGCAATAATAAATCCACTCTC
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TTAACCAGTCGCTAGATGCCCTTCTGTCCAGGGACTTGATCATGAAAGA
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CAATTACTAGACACTACTGACATCCAAGGAGAAGAATTTGCCAAAGTTA
TAGTACAAAAATTGAAAGATAACAAACAAATGGGTCTTCAGCCTTACCC
GGAAATACTTGTGGTTTCTAGATCACCATCTTTAAATTTACTTCAAAAT
AAAAGCATGTAAgcggccgc