

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RIPK2 [2 – 311]

<u>Enzyme description:-</u>	RIPK2 [2 – 311]
<u>Clone number:-</u>	DU 16348
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus Expression Vector System
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	63, 537.71 daltons
Average Mass	63, 578.44 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.25
<u>Purity:-</u>	>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

Assay: Standard Filter Binding

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM₂MgAc

Substrate:-

Myelin Basic Protein Final concentration: 0.3 mg/ml

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Clone Data Sheet

RIPK2 [2 – 311]

<u>Protein</u>	RIPK2 [2 – 311]
<u>Clone number</u>	DU 63057
<u>Species</u>	Human
<u>Accession number</u>	NM_003821
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSPEFPGRLERPNGEA ICSALPTIPYHKLADLRYLSRGASGTVSSARHADWRVQVAVKHLHIHTP LLDSERKDVLR AEILHKARFSYILPILGICNEPEFLGIVTEYMPNGSL NELLHRKTEYPDVAWPLRFRILHEIALGVNYLHNMTPLLHHDLKTQNI LLDNEFHVKIADFGLSKWRMMSLSQSRSSKSAPEGGTIIYMPENYEPG QKSRASIKHDIYSYAVITWEVLSRKQPFEDVTNPLQIMYSVSQGHRPVI NEESLPYDIPHRARMISLIESGWAQNPDERPSFLKCLIELEPVLRTFEE ITFLEAVIQLKK</p>
<u>Native sequence</u>	<p>Amino acids N2 – K311 (full length is M540) of human RIPK2. Residue N242 of the fusion protein is equivalent to N2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Not1</i> sites into pFastBacDual GST

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Nucleotide Sequence:

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGGGAATATCTTGAAGAAAAATATGAAGAGCATTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCATTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATAACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGCCCTGGGATCCCCGGAAT
TCCCGGGTCGACTCGAGCGGCCGAACGGGGAGGCCATCTGCAGCGCCCTG
CCCACCATTCCCTACCACAACTCGCCGACCTGCGCTACCTGAGCCGCGG
CGCCTCTGGCACTGTGTCTCGCCCGCCACGCAGACTGGCGCGTCCAGG
TGGCCGTGAAGCACCTGCACATCCACACTCCGCTGCTCGACAGTGAAAGA
AAGGATGTCTTAAGAGAAGCTGAAATTTTACACAAAGCTAGATTTAGTTA
CATTCTTCCAATTTTGGGAATTTGCAATGAGCCTGAATTTTGGGAATAG
TTACTGAATACATGCCAAATGGATCATTAATGAACCTCCTACATAGGAAA
ACTGAATATCCTGATGTTGCTTGGCCATTGAGATTTTCGCATCCTGCATGA
AATTGCCCTTGGTGTAAATTACCTGCACAATATGACTCCTCCTTTACTTC
ATCATGACTTGAAGACTCAGAATATCTTATTGGACAATGAATTTTCATGTT
AAGATTGCAGATTTTGGTTTATCAAAGTGGCGCATGATGTCCCTCTCACA
GTCACGAAGTAGCAAATCTGCACCAGAAGGAGGGACAATTATCTATATGC
CACCTGAAAACCTATGAACCTGGACAAAAATCAAGGGCCAGTATCAAGCAC
GATATATATAGCTATGCAGTTATCACATGGGAAGTGTATCCAGAAAACA
GCCTTTTGAAGATGTCACCAATCCTTTGCAGATAATGTATAGTGTGTCAC
AAGGACATCGACCTGTTATTAATGAAGAAAGTTTGCCATATGATATACCT
CACCGAGCACGTATGATCTCTCTAATAGAAAGTGGATGGGCACAAAATCC
AGATGAAAGACCATCTTCTTAAAATGTTTAAATAGAACTTGAACCAGTTT
TGAGAACATTTGAAGAGATAACTTTTCTTGAAGCTGTTATTCAGCTAAAG
AAAtaa

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