Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RIPK2 [2 - 311]

Enzyme description:- RIPK2 [2 - 311]

Clone number:- DU 16348

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 63, 537.71daltons
Average Mass 63, 578.44 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.25

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Myelin Basic Protein Final concentration: 0.33 mg/ml

Division of Signal Transduction Therapy

Clone Data Sheet

RIPK2 [2 - 311]

Protein	RIPK2 [2 - 311]
Clone number	DU 16348
Species	Human
Accession number	NM_003821
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFPGRLER P**NGEAICSALPTIPYHKLADLRYLSRGASGTVSSARHADWRVQVAVKH** **LHIHTPLLDSERKDVLREAEILHKARFSYILPILGICNEPEFLGIVTE** **YMPNGSLNELLHRKTEYPDVAWPLRFRILHEIALGVNYLHNMTPPLLH** **HDLKTQNILLDNEFHVKIADFGLSKWRMMSLSQSRSSKSAPEGGTIIY** **MPPENYEPGQKSRASIKHDIYSYAVITWEVLSRKQPFEDVTNPLQIMY** **SVSQGHRPVINEESLPYDIPHRARMISLIESGWAQNPDERPSFLKCLI** **ELEPVLRTFEEITFLEAVIQLKK**
Native sequence	Amino acids N2 – K311 of human RIPK2. [Full length protein ends at residue M540] Residue N242 of the fusion protein is equivalent to N2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Not*1 sites of pFB GST 6P-1

Nucleotide sequence of insert

gcggccgAACGGGGAGGCCATCTGCAGCGCCCTGCCCACCATTCCCTA
CCACAAACTCGCCGACCTGCGCTACCTGAGCCGCGGCGCCTCTGGCAC
TGTGTCGTCCGCCCGCCACGCAGACTGGCGCGTCCAGGTGGCCGTGAA
GCACCTGCACATCCACACTCCGCTGCTCGACAGTGAAAGAAAGGATGT
CTTAAGAGAAGCTGAAATTTTACACAAAGCTAGATTTAGTTACATTCT
TCCAATTTTGGGAATTTGCAATGAGCCTGAATTTTTGGGAATAGTTAC
TGAATACATGCCAAATGGATCATTAAATGAACTCCTACATAGGAAAAC
TGAATATCCTGATGTTGCTTGGCCATTGAGATTTCGCATCCTGCATGA
AATTGCCCTTGGTGTAAATTACCTGCACAATATGACTCCTCCTTTACT
TCATCATGACTTGAAGACTCAGAATATCTTATTGGACAATGAATTTCA
TGTTAAGATTGCAGATTTTGGTTTATCAAAGTGGCGCATGATGTCCCT
CTCACAGTCACGAAGTAGCAAATCTGCACCAGAAGGAGGGACAATTAT
CTATATGCCACCTGAAAACTATGAACCTGGACAAAAATCAAGGGCCAG
TATCAAGCACGATATATATAGCTATGCAGTTATCACATGGGAAGTGTT
ATCCAGAAAACAGCCTTTTGAAGATGTCACCAATCCTTTGCAGATAAT
GTATAGTGTGTCACAAGGACATCGACCTGTTATTAATGAAGAAAGTTT
GCCATATGATATACCTCACCGAGCACGTATGATCTCTCTAATAGAAAG
TGGATGGGCACAAAATCCAGATGAAAGACCATCTTTCTTAAAATGTTT
AATAGAACTTGAACCAGTTTTGAGAACATTTGAAGAGATAACTTTTCT
TGAAGCTGTTATTCAGCTAAAGAAAtaa