Certificate of Analysis
RIPK2 , 1 mg

Recombinant Human Receptor Interacting Serine Threonine Kinase 2, Histidine-tagged

Part Number: PV4325
Lot Number: 35334K
Immediate Storage: -80°C
Shipping Conditions: dry ice

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Description:

Recombinant Human protein, Catalytic Domain (amino acids 1-299), His-tagged, expressed in insect cells. No special measures were taken to activate this kinase.

Manufacturing:

Manufactured under ISO 9001 certification at Life Technologies in Madison, WI, USA.

Specific Activity:

37 nmoles of phosphate transferred to myelin basic protein (MBP) per minute per mg of total protein at 30°C. Activity determined at a final protein concentration of 16.7 µg/mL.

Concentration:

0.47 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **12,100 nM**.

Aliases:

RICK, RIP2, CARD3, CARDIAK

Storage and Handling:

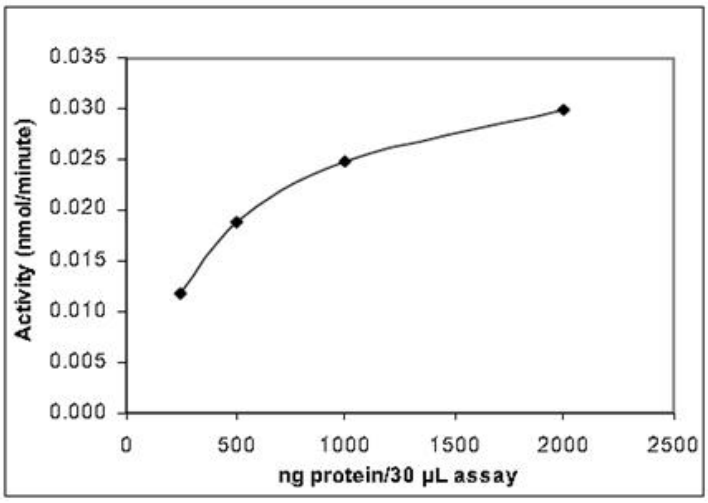
For maximum recovery please spin prior to use. Aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at −80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

50 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.02% Triton® X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

RIPK2 Activity Graph



Gel Information for RIPK2

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex® gels (Catalog #: EC6025BOX).

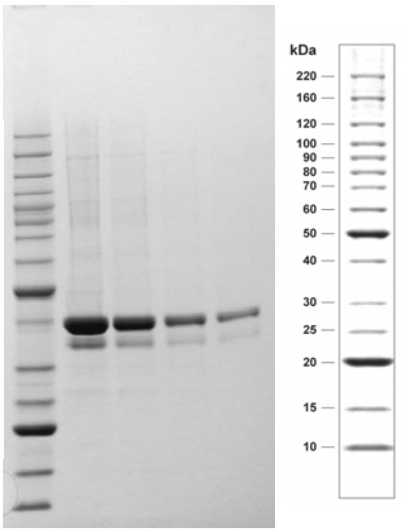
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 10 µg RIPK2

Lane 3: 5 µg RIPK2

Lane 4: 2 µg RIPK2

Lane 5: 1 µg RIPK2



Dilution Buffer:

20 mM Tris (pH 7.5), 0.02% Triton® X−100, 0.1 mg/mL BSA, 2 mM DTT, 0.5 mM Na_3VO_4 and 10% Glycerol.

Assay Conditions:

RIPK2 was pre-diluted in enzyme dilution buffer and assayed in 25 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 5 mM β−glycerophosphate, 2.5 mM DTT, 0.01% Triton® X–100, 200 µM ATP, 500 µg/mL myelin basic protein (MBP) and trace [^{32}P]-γ-ATP for 10 minutes at 30°C.

Purity:

75% as determined by a Coomassie® blue stained SDS-PAGE gel.

Molecular Weight:

38.9 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:

RIPK2 was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified RIPK2 identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_003812

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   1 MSYYHHHHHH DYDIPTTENL YFQGITSLYK KAGFEGDSTM NGEAICSALP TIPYHKLADL RYLSRGASGT VSSARHADWR VQVAVKHLHI HTPLLDSERK IVGN RIPK2
   1 ---------- ---------- ---------- ---------M NGEAICSALP TIPYHKLADL RYLSRGASGT VSSARHADWR VQVAVKHLHI HTPLLDSERK NP_003812

 101 DVLREAEILH KARFSYILPI LGICNEPEFL GIVTEYMPNG SLNELLHRKT EYPDVAWPLR FRILHEIALG VNYLHNMTPP LLHHDLKTQN ILLDNEFHVK
  62 DVLREAEILH KARFSYILPI LGICNEPEFL GIVTEYMPNG SLNELLHRKT EYPDVAWPLR FRILHEIALG VNYLHNMTPP LLHHDLKTQN ILLDNEFHVK

 201 IADFGLSKWR MMSLSQSRSS KSAPEGGTII YMPPENYEPG QKSRASIKHD IYSYAVITWE VLSRKQPFED VTNPLQIMYS VSQGHRPVIN EESLPYDIPH
 162 IADFGLSKWR MMSLSQSRSS KSAPEGGTII YMPPENYEPG QKSRASIKHD IYSYAVITWE VLSRKQPFED VTNPLQIMYS VSQGHRPVIN EESLPYDIPH

 301 RARMISLIES GWAQNPDERP SFLKCLIELE PVLRTFEE.
 262 RARMISLIES GWAQNPDERP SFLKCLIELE PVLRTFEE
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* highlighted residues denote differences from the reference protein sequence(s).

Marlene Johnson, Manager, QA/QC Date: 26/Mar/2012