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Certificate of Analysis

RIPK1, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 16-022, 16-022-K, 16-022M
Parent Lot # D16KP002N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *C*-terminal 6His-tagged, recombinant, human RIPK1 amino acids 8-322 co-expressed with untagged human CDC37, full length by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 37kDa.

Specific Activity (Parent lot# D16KP002N): 7U/mg, where one unit of RIPK1 activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.99mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

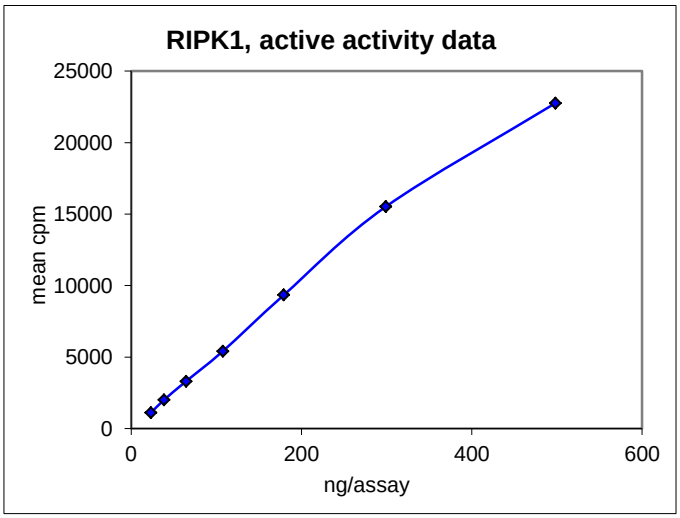
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

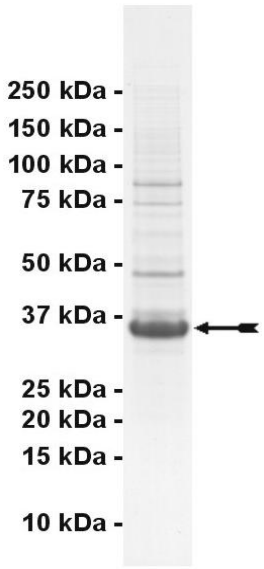
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 23–498ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as RIPK1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of RIPK1, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom

T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Myelin basic protein**: Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **Manganese chloride**: Use at a final assay concentration of 10mM. Prepare a 100mM stock and use 2.5µl per assay point.
4. **RIPK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 23–498ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of myelin basic protein.
3. Add **2.5µl (23–498ng) RIPK1, active.**
4. Add 2.5µl of 100mM manganese chloride
5. Add 2.5µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 30 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the dried filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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RIPK1, active Sequence Information

Protein	Human RIPK1
Tags	*C*-terminal 6His
Native sequence	N2 of the recombinant protein is equivalent to N8 of human RIPK1
Accession number	GenBank NM_003804.3

Recombinant RIPK1 amino acid sequence:

```
  1 MNVIKMKSSD FLESAELDSG GFGKVSLCFH RTQGLMIMKT VYKGPNCIEH NEALLEEAKM
 61 MNRLRHSRVV KLLGVIIEEG KYSLVMEYME KGNLMHVLKA EMSTPLSVKG RIILEIIEGM
121 CYLHGKGVIH KDLKPENILV DNDFHIKIAD LGLASFKMWS KLNNEEHNEL REVDGTAKKN
181 GGTLYYMAPE HLNDVNAKPT EKSDVYSFAV VLWAIFANKE PYENAICEQQ LIMCIKSGNR
241 PDVDDITEYC PREIISLMKL CWEANPEARP TFPGIEEKFR PFYLSQLEES VEEDVKSLKK
301 EYSNENAVVK RMQSLQGPGP GHHHHHH
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Recombinant RIPK1 nucleotide sequence:

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  1 atgaatgtca ttaagatgaa atccagtgac ttcctggaga gtgcagaact ggacagcgga
 61 ggcttcggga aggtgtctct gtgtttccac agaacccagg gactcatgat catgaaaaca
121 gtgtacaagg ggcccaactg cattgagcac aacgaggccc tcttggagga ggcgaagatg
181 atgaacagac tgagacacag ccgggtggtg aagctcctgg gcgtcatcat agaggaaggg
241 aagtactccc tggtgatgga gtacatggag aagggcaacc tgatgcacgt gctgaaagcc
301 gagatgagta ctccgctttc tgtaaaagga aggataattt tggaaatcat tgaaggaatg
361 tgctacttac atggaaaagg cgtgatacac aaggacctga agcctgaaaa tatccttgtt
421 gataatgact tccacattaa gatcgcagac ctcggccttg cctcctttaa gatgtggagc
481 aaactgaata atgaagagca caatgagctg agggaagtgg acggcaccgc taagaagaat
541 ggcggcaccc tctactacat ggcgcccgag cacctgaatg acgtcaacgc aaagcccaca
601 gagaagtcgg atgtgtacag ctttgctgta gtactctggg cgatatttgc aaataaggag
661 ccatatgaaa atgctatctg tgagcagcag ttgataatgt gcataaaatc tgggaacagg
721 ccagatgtgg atgacatcac tgagtactgc ccaagagaaa ttatcagtct catgaagctc
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841 ccttttttatt taagtcaatt agaagaaagt gtagaagagg acgtgaagag tttaaagaaa
901 gagtattcaa acgaaaatgc agttgtgaag agaatgcagt ctcttcaagg cccgggccct
961 ggccatcacc atcaccatca ctaa
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Reviewed and approved by site quality representative.