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Certificate of Analysis

RIPK2 Kinase, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-612, 14-612-K, 14-612M
Parent Lot # 2073338

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: *N*-terminal 6His-tagged, recombinant, human RIPK2, amino acids 1–299, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 76.6% by SDS-PAGE and Coomassie blue staining. MW = 37.8kDa.

Specific Activity (Parent lot# 2073338): 54U/mg, where one unit of RIPK2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.097mg/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.

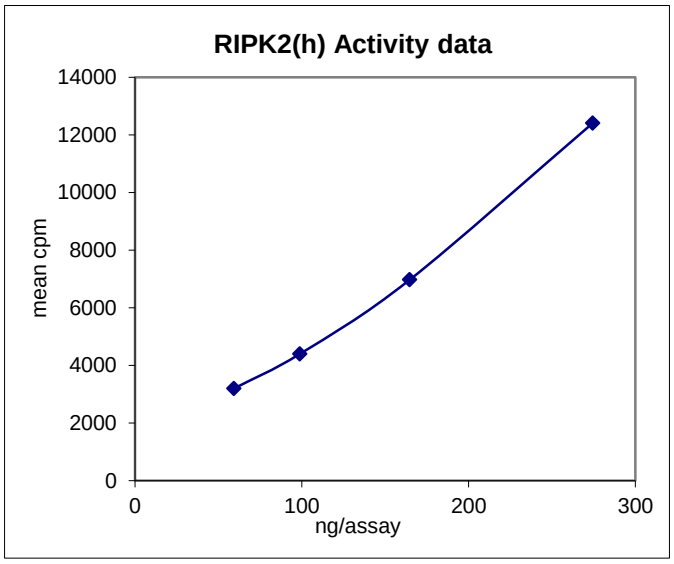
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

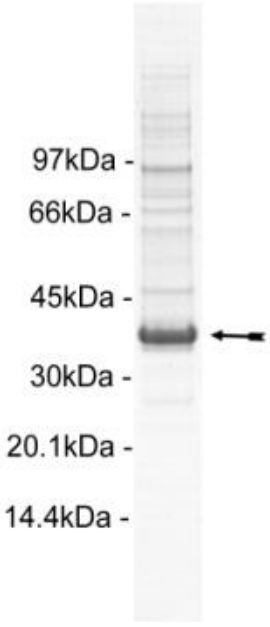
Quality Control Testing

Kinase Assay: 59–274ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) 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 RIPK2 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 RIPK2, active.



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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 (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **RIPK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 59–274ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[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 (MBP)**.
3. Add **2.5µl (59–274ng) RIPK2, active**.
4. Add 5µl of dH_2O.
5. Add 10 µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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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RIPK2 Sequence Information

Protein human RIPK2

Tags *N*-terminal 6His

Native sequence M29 of the fusion protein is equivalent to M1 of human RIPK2

Accession number GenBank NM_003821

Recombinant RIPK2 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMN GEAICSALPT IPYHKLADLR YLSRGASGTV
 61 SSARHADWRV QVAVKHLHIH TPLLDSERKD VLREAEILHK ARFSYILPIL GICNEPEFLG
121 IVTEYMPNGS LNELLHRKTE YPDVAWPLRF RILHEIALGV NYLHNMTPPL LHHDLKTQNI
181 LLDNEFHVKI ADFGLSKWRM MSLSQSRSSK SAPEGGTIIY MPPENYEPGQ KSRASIKHDI
241 YSYAVITWEV LSRKQPFEDV TNPLQIMYSV SQGHRPVINE ESLPYDIPHR ARMISLIESG
301 WAQNPDERPS FLKCLIELEP VLRTFEE
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Recombinant RIPK2 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atctatgaac ggggaggcca tctgcagcgc cctgcccacc
121 attccctacc acaaactcgc cgacctgcgc tacctgagcc gcggcgcctc tggcactgtg
181 tcgtccgccc gccacgcaga ctggcgcgtc caggtggccg tgaagcacct gcacatccac
241 actccgctgc tcgacagtga aagaaaggat gtcttaagag aagctgaaat tttacacaaa
301 gctagattta gttacattct tccaattttg ggaatttgca atgagcctga atttttggga
361 atagttactg aatacatgcc aaatggatca ttaaatgaac tcctacatag gaaaactgaa
421 tatcctgatg ttgcttggcc attgagattt cgcatcctgc atgaaattgc ccttggtgta
481 aattacctgc acaatatgac tcctccttta cttcatcatg acttgaagac tcagaatatc
541 ttattggaca atgaatttca tgttaagatt gcagattttg gtttatcaaa gtggcgcatg
601 atgtccctct cacagtcacg aagtagcaaa tctgcaccag aaggagggac aattatctat
661 atgccacctg aaaactatga acctggacaa aaatcaaggg ccagtatcaa gcacgatata
721 tatagctatg cagttatcac atgggaagtg ttatccagaa aacagccttt tgaagatgtc
781 accaatcctt tgcagataat gtatagtgtg tcacaaggac atcgacctgt tattaatgaa
841 gaaagtttgc catatgatat acctcaccga gcacgtatga tctctctaat agaaagtgga
901 tgggcacaaa atccagatga aagaccatct ttcttaaaat gtttaataga acttgaacca
961 gttttgagaa catttgaaga gtag
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Reviewed and approved by site quality representative.