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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
R07M-11G -05	5 µg
R07M-11G -10	10 µg

RIPK1, Active

Recombinant mouse protein expressed in Sf9 cells

Catalog # R07M-11G

Lot # V2253-5

Product Description

Recombinant mouse RIPK1 (1-327) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The RIPK1 gene accession number is NM_009068.

Gene Aliases

D330015H01Rik; Rinp; RIP; Rip1

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

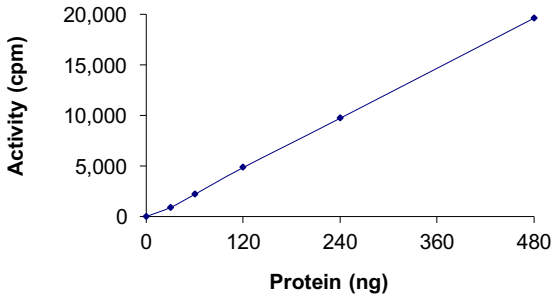
Scientific Background

RIPK1 or Receptor Interacting Protein Kinase 1 is a serine/threonine kinase that was originally identified as interacting with the cytoplasmic domain of FAS. RIPK1 has been deemed as an important element in the signal transduction machinery that mediates programmed cell death. RIPK1 has been shown to interact with TRADD, TRAF1, TRAF2 and TRAF3 and TRADD can act as an adaptor protein to recruit RIPK1 to the TNFR1 complex in a TNF-dependent process (1). TNFa is capable of activating the noncanonical NF-κB pathway, but this activation of this pathway is negatively regulated by RIPK1 (2).

References

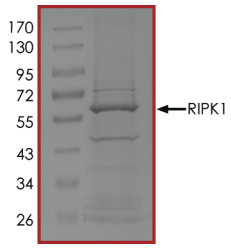
1. Hsu, H. et al: TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4: 387-396, 1996.
2. Kim, J Y. et al: TNFa induced noncanonical NF-κB activation is attenuated by RIP1 through stabilization of TRAF2. J Cell Sci. 2011 Feb 15;124(Pt 4):647-56.

Specific Activity



The specific activity of RIPK1 was determined to be **2.6 nmol /min/mg** as per activity assay protocol.

Purity



The purity of RIPK1 was determined to be **>70%** by densitometry, approx. MW **62 kDa**.

RIPK1, Active

Recombinant mouse protein expressed in Sf9 cells

Catalog #	R07M-11G
Specific Activity	2.6 nmol/min/mg
Lot #	V2253-5
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: R07M-11G)

Active mouse RIPK1 (0.1μg/μl) diluted with Kinase Dilution Buffer VI (Catalog #: K24-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active RIPK1 for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: M42-51N)

MBP Protein diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active RIPK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active RIPK1 (Catalog #R07M-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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