

Catalog #	Aliquot Size
R09-10G -05	5 µg
R09-10G -10	10µg

RIPK3, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # R09-10G

Lot # K1648-5

Product Description

Recombinant full-length human RIPK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The RIPK3 gene accession number is [NM_006871](#).

Gene Aliases

RIP3

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.

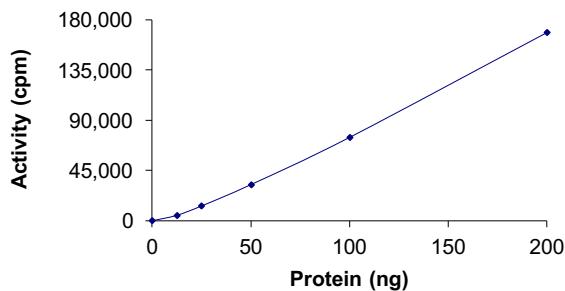
Scientific Background

RIPK3 is a member of the receptor-interacting protein (RIP) family of serine/threonine protein kinases, which contains a C-terminal domain unique from other RIP family members. RIPK3 is predominantly localized to the cytoplasm, and specifically, are colocalized in the mitochondrion which can undergo nucleocytoplasmic shuttling dependent on novel nuclear localization and export signals (1). RIPK3 is a component of the tumor necrosis factor (TNF) receptor-I signaling complex, and can induce apoptosis and weakly activate the NF-kappaB transcription factor and caspase-8 prevents RIPK3-dependent necrosis without inducing apoptosis by functioning in a proteolytically active complex with CFLAR and that this complex is required for the protective function (2).

References

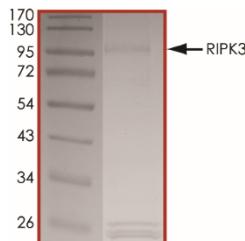
1. Kasof, G. M. et.al: The RIP-like kinase, RIP3, induces apoptosis and NF-kappa-B nuclear translocation and localizes to mitochondria. FEBS Lett. 473: 285-291, 2000.
2. Oberst, A. et.al: Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 471: 363-367, 2011.

Specific Activity



The specific activity of RIPK3 was determined to be **36 nmol /min/mg** as per activity assay protocol.

Purity



The purity of RIPK3 was determined to be **>70%** by densitometry, approx. MW **96 kDa**.

RIPK3, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	R09-10G
Specific Activity	36 nmol/min/mg
Lot #	K1648-5
Purity	>70%
Concentration	0.05 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 #: R09-10G)

Active RIPK3 (0.05 μ g/ μ l) diluted with Kinase Dilution Buffer IV (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 RIPK3 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/ μ l BSA solution.

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

Buffer components: 25mM MOPS pH 7.2, 12.5mM β -glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 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 200 μ l aliquots at -20°C.

Substrate (Catalog #: M42-51N)

MBP Protein substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active RIPK3, 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 20 μ l:
 - Component 1. 10 μ l of diluted Active RIPK3 (Catalog #R09-10G)
 - Component 2. 5 μ l of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3. 5 μ l distilled H₂O (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 H₂O.
- Step 5. Initiate the reaction by the addition of 5 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l 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 μ l 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

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

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

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