

Catalog #	Aliquot Size
R08-16G-20	20 µg
R08-16G-50	50 µg

RIPK2 (K47A), Unactive

Recombinant human protein expressed in Sf9 cells

Catalog # R08-16G

Lot # B2117-9

Product Description

Recombinant human RIPK2 (K47A) (1-299) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_003821](#).

Gene Aliases

RICK; RIP2; CARD3; CARDIAK

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.

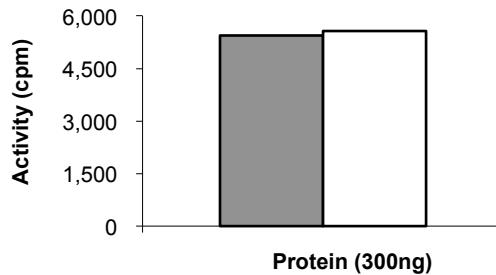
Scientific Background

RIPK2 (RIP2; RICK) is a death domain-containing protein kinase encoding with predicted 540-amino acid protein. It contains an N-terminal serine/threonine kinase catalytic domain and a C-terminal caspase activation and recruitment domain. RIPK2 is thought to regulate apoptosis induced by the FAS receptor pathway (1). RIPK2 has been shown to specifically interact with the CARD of ICE/caspase-1 and this interaction correlates with the processing of pro-caspase-1 and the formation of the active caspase-1 p20 (2).

References

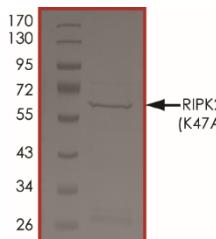
- Inohara, N. et al: RICK, a novel protein kinase containing a caspase recruitment domain, interacts with CLARP and regulates CD95-mediated apoptosis. *J. Biol. Chem.* 273: 12296-12300, 1998. Note: Erratum: *J. Biol. Chem* 273: 18675 only, 1998.
- Thome, M. et al: Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. *Curr. Biol.* 8: 885-888, 1998.

Specific Activity



Kinase-dead RIPK2 (K47A) activity assay with or without protein substrate MBP. This recombinant Kinase-dead RIPK2 mutant did not show significant kinase activity *in vitro* using the activity assay protocol in the data sheet.

Purity



The purity of RIPK2 (K47A) was determined to be >80% by densitometry.
Approx. MW 59kDa.

RIPK2 (K47A), Unactive

Recombinant human protein expressed in Sf9 cells

Catalog Number R08-16G

Lot # B2117-9

Purity >80%

Concentration 0.05µg/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 #: R08-16G)

Unactive RIPK2 (K47A) (0.05 μ g/ μ l) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 Unactive RIPK2 (K47A) for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/ μ l BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 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 I (Catalog #: K01-09). 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 I (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) 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 Unactive RIPK2 (K47A), 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 Unactive RIPK2 (K47A) (Catalog #R08-16G)
 - 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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