

Product Information

RIPK3, active, GST-tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number **SRP5316**

Storage Temperature –70 °C

Synonyms: RIP3

Product Description

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 are colocalized in the mitochondria, and 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-κB transcription factor. Caspase-8 prevents RIPK3-dependent necrosis without inducing apoptosis by functioning in a proteolytically active complex with CFLAR and this complex is required for the protective function.²

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. It is supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~96 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.

SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

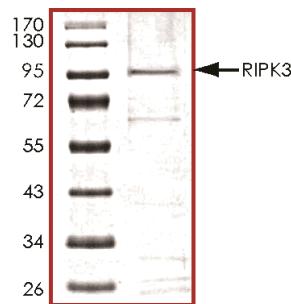
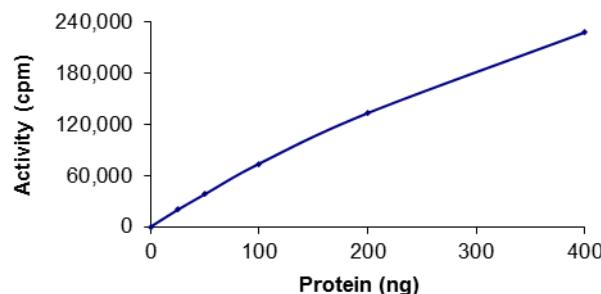


Figure 2.

Specific Activity of Typical Lot:
30–51 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS pH 7.2, 12.5 mM glycerol 2-phosphate, 20 mM MgCl₂, 12.5 mM MnCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/μL BSA solution.

Kinase Solution – Dilute the active RIPK3 (0.05 µg/µL) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active RIPK3 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 mL of Kinase Assay Buffer. Store in 200 µL aliquots at –20 °C.

γ -³³P-ATP Assay Cocktail (250 µM) – Combine 5.75 mL of Kinase Assay Buffer, 150 µL of 10 mM ATP Stock Solution, 100 µL of γ -³³P-ATP (1 mCi/100 µL). Store in 1 mL aliquots at –20 °C.

Substrate Solution – MBP Protein substrate diluted in distilled water to a final concentration of 1 mg/mL.

1% phosphoric acid solution – Dilute 10 mL of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active RIPK3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³³P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µL:
10 µL of Kinase Solution
5 µL of Substrate Solution
5 µL of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µL of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µL of the γ -³³P-ATP Assay Cocktail, bringing the final reaction volume to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µL of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ -³³P-ATP counts introduced into the reaction. Spot 5 µL of the γ -³³P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{L of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 µL of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δ cpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Kasof, G. M. et al., The RIP-like kinase, RIP3, induces apoptosis and NF- κ 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).

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