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Product Specification

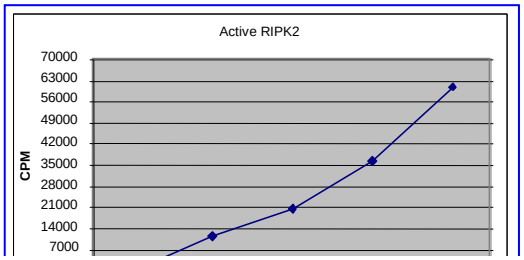
RIPK2, active
(Recombinant human protein, residues 1-299, expressed in Sf 9 cells)

Catalog #: 7747-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 20 nmol/min/mg

Quality Control Analysis
Activity assessment
RIPK2 protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the MBP in the following assay condition:

10 µl diluted RIPK2 protein
10 µl MBP (1 mg/ml stock)
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.



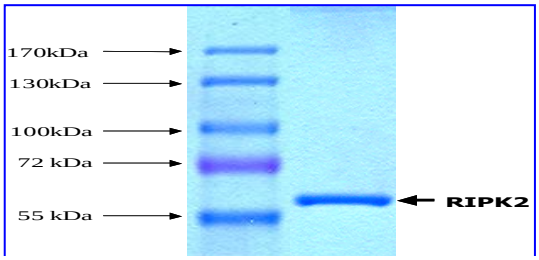


Fig. 1 RIPK2 activity assay

Fig. 2 RIPK2 protein gel

Purity assessment
1 µg of protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the RIPK2 product, and the band was at ~59 kDa (Fig. 2).

Product Description

Recombinant human RIPK2 (1-299) containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_003821.
This material is sold for research purposes only.

Specific Activity
20 nmol phosphate incorporated into MBP per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
RIPK2 (RIP2; RICK) is a death domain-containing protein kinase. Inohara identified cDNAs encoding a predicted 540-amino acid protein RICK, which contains an N-terminal serine/threonine kinase catalytic domain and a C-terminal caspase activation and recruitment domain. Inohara also demonstrated that RICK is a novel kinase that may regulate apoptosis induced by the FAS receptor pathway (1). McCarthy found that overexpression of RIP2 signaled both cell death and NF-kappa-B activation (2). Thome reported that RICK specifically interacted with the CARD of ICE/caspase-1, and this interaction correlated with the processing of pro-caspase-1 and the formation of the active caspase-1 p20 (3). Chin generated Ripk2-deficient mice and concluded that RIPK2 is implicated in the innate response to pathogens by NOD and TLR-induced cell signaling and mediates cytokine-induced Ifng production in Th1 and NK cells (4). Also Kobayashi demonstrated that RIPK2 is required for signaling through both TLR and NOD protein family members in the innate immune system as well as for appropriate TCR signaling in the adaptive immune response (5).

References
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