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New Item

New Item

Serine Threonine kinases selective antibodies

Receptor-Interacting Serine/threonine-protein kinase 3 (RIP3 kinase) Catalog # RIP3-101AP, P-RIP and PC-RIP3

Other nomenclature: RIPk3;

Receptor interacting serine/threonine kinase protein 3 (RIP3) is a member of the RIP kinase family. It is expressed in the embryo and in multiple adult tissues, including most hemopoietic cell lineages. RIP3 is a member of the RIP kinase family which is believed to be dispensable for normal development of mouse embryos (1). The RIP3 deficient cells exhibit normal sensitivity to a variety of apoptotic stimuli and were indistinguishable from wild type cells in their ability to activate NF-kB signaling in response to the TNFF and LPS (1). What role if any RIP3 plays in the regulation of apoptosis and N-kB signaling by activation or attenuation of NF-kB family of transcription factors is not clearly understood and still controversial. RIP3 interacts with serine/threonine kinase RIP with an N-terminal kinases domain as found in RIP and RIP2 kinases. The RIP posses C-terminal death domain, while RIP2 has a C-terminal caspase activation domain and RIP3 has a unique C-terminus. The murine herpesviruses such as cytomegalovirus M45 protein interacts with receptor-interacting protein (RIP) 1 and RIP3 via a RIP homotypic interaction motif.

Programmed necrosis is a form of caspase-independent cell death whose molecular regulation is poorly understood. The kinase RIP1 is crucial for programmed necrosis, but also mediates activation of the prosurvival transcription factor NF-kappaB. The necrosis can be induced by stimulating death receptors with TNF or other death receptor agonists. The RIP3 is the molecular switch that is required for necrosis in NIH 3T3 cells followed by TNF treatment. RIP3 did not affect RIP1 mediated apoptosis but was required for RIP1 mediated necrosis. RIP3 also regulates TNF-induced reactive oxygen species production that partially accounts for RIP3's ability to promote necrosis (2). RIP3 regulates necrosis-specific RIP1 phosphorylation, and the phosphorylation of RIP1 and RIP3 stabilizes their association within the pronectrotic complex, that activates the pronecrtic kinase activity that triggers the downstream reactive oxygen species production. RIP3 controls programmed necrosis by initiating the pronecrotic kinases cascade and is necessary for the inflammatory response against viral infections (3). RIP3 is a nucleocytoplasmic shuttling protein and its unconventional nuclear localization signal (NLS, 442-472 aa) is sufficient to trigger apoptosis in the nucleus in many cells. This NLS motif is involved in RIP-RIP3 interaction, is necessary for RIP3 self association and for RIP3 induced apoptosis and RIP3 induced NF-kB activation (4). Several splice variant of this protein has been sequences based on conceptual translation.

The RIPk3 protein is a approximately 518 amino acid (64kDa) protein. FabGennix has made an antibody to RIP3 protein using antigeninc peptide methodology. The RIP3-selective antibody was generated against a peptide from the unique C-terminal end of RIP3 protein. The anti-RIP3 antibodies are affinity purified against immobilized antigen based affinity matrix. The purified mono-specific polyclonal antibodies strongly labels two bands at 74 and 64 kDa in RIP3 western blot positive controls (PC-RIP3). *FabGennix Inc*. will also conjugate antibodies with fluorescent probes asnd secondary enzymes upon request at a nominal charge. Most of our catalog antibodies are available in FITC conjugated form for direct application in IHC. *FabGennix Inc.* has made a number of antibodies to various kinases, for a complete listing please visit www.fabgenni.com. FabGennix, Inc., will also provide Western blot positive control in ready-to-use buffer for easy identification of RIP3 along with antigenic blocking peptide for competition assays. Please enquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	volume
RIP3-301AP	Rabbit	Affinity purified RIP3 antibody	H, monk	100 l	200ul
FITC-RIP3	Rabbit	FITC conjugated RIP3 antibody	H,monk	100 g	200ul
P-RIP3	Rabbit	Antigenic blocking peptide for RIP3-301AP	n/a	250 ug	100ul
PC-RIP3	cells	Western blot positive control for RIP3	H	5 appl	inquire

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; * not all variants are labeled equally

Immunogen: Synthetic peptide selected from amino acids 498-518corresponding to the following sequence (egp kdp pea wsr pqg wyn hsg k),was selected from the unique region near C-terminal end of RIP3protein, peptide was post-synthetically modified to achieve highest antigenicty before used for coupling to KLH using heterobifunctional cross linker for immunogen preparation.

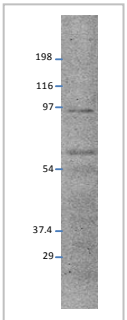
Concentration: RIP3-301AP101AP IgG concentration 0.66-0.68 mg/ml.

Applications: Antibody RIP3-301AP is ideal for IMM and WB. RIP3-131AP has not been tested in other applications. The dilutions for these antibodies are for reference only, investigators are expected to determine the optimal conditions for specific assay. WB; 1:500; IMM & i.p pull-down assays:> 1:200 (1 ul/250 ug protein extracts). Investigators interested in using this antibody for applications other than listed here can request for a complimentary smaple.

Reactivity: This antibody detects 2 bands corresponding to 74 and 64kDa in PC-RIP3 samples. This antibody will labels all the 5 isoform/variants of RIP3 in cells.

Protocols: Standard protocol for various applications (WB, IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Int. Inc.

Form/Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide. For long-term storage of antibodies, store at -20°C. FabGennix Int. Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DilUbuffer). Working solutions of antibodies in DilUbuffer should be filtered through 0.45□ filter after every use for long-term storage.



Western blot of RIP3 with RIP3-301AP (1:500) in diluObuffer.

References:

1. Kim Newton, Xiaoqing Sun, and Vishva M. Dixit. Kinase RIP3 Is Dispensable for Normal NF-κBs, Signaling by the B-Cell and T-Cell Receptors, Tumor Necrosis Factor Receptor 1, and Toll-Like Receptors 2 and 4. Mol Cell Biol. 2004 February; 24(4): 1464–1469.
2. Jason W. Upton, William J. Kaiser, and Edward S. Mocarski. Cytomegalovirus M45 Cell Death Suppression Requires Receptor-interacting Protein (RIP) Homotypic Interaction Motif (RHIM)-dependent Interaction with RIP1*. J Biol Chem. 2008 June 20; 283(25): 16966–16970.
3. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. 1: Science. 2009 Jul 17;325(5938):332-6. Epub 2009 Jun 4.
4. Li M, Feng S, Wu M. Multiple roles for nuclear localization signal (NLS, aa 442-472) of receptor interacting protein 3 (RIP3). Biochem Biophys Res Commun. 2008 Aug 8;372(4):850-5. Epub 2008 Jun 3.

*For users who may require large amounts of RIP3-301AP and FITC-RIP3, please enquire about bulk material discounts.

This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

101209-0020SF1001Z-rev10.00