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Antibodies to GTP binding proteins

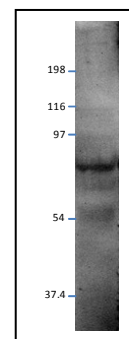
Antibodies to Synembryn or Ric8A (Catalog # Ric8A-101AP, P-Ric8A and PC-Ric8A)

Other Nomenclature: *Synembryn*; heterotrimeric G protein guanine nucleotide exchange factor Ric-8A; resistance to inhibitors of cholinesterase 8A. Resistance to inhibitors of cholinesterase (Ric) 8A is a guanine nucleotide exchange factor that activates certain G protein alpha-subunits. Earlier genetic studies have put RIC-8 under uncharacterized G protein signaling pathway that regulates centrosome movements during cell division. Various components of this pathway include G protein subunits of the Galphai class, GPR or GoLoco domain-containing proteins, RGS (regulator of G protein signaling) proteins, and other accessory factors. These proteins interact to regulate microtubule pulling forces during mitotic movement of chromosomes. The nucleus and the mitochondria of eukaryotic cells, as well as the chloroplasts in plants, contain their own genome that encodes a range of proteins and nucleic acids. The mitochondrial tRNA is encoded by nucleus and is transported into mitochondria in many species with the help of mitochondrial transport complex as described (1). RIC8A is a 21-kDa tRNA-binding component of the tRNA complex that is partly homologous to subunit UCR6b of ubiquinol-cytochrome c reductase (OX PHOS complex III). RIC8A has binding specificity for the so-called type II tRNAs which, *in vitro*, require stimulation by a second group of tRNAs (type I) for import across the inner mitochondrial membrane; type I tRNAs, on the other hand, are imported efficiently by themselves, but this import is inhibited by type II tRNAs. Knockdown of RIC8A leads to deficiency of mitochondrial type II and elevation of type I tRNAs *in vivo* (2). In the presence of ATP, both types of tRNA are transferred from their respective receptors to a third subunit, RIC9, and thence through the membrane (3). Ric8A also interacts with Adenylyl cyclase 5A to regulate its activity. The N-terminus of each individual AC usually has a unique amino acid sequence that is important for isoform-specific regulation (3). Several interacting proteins [e.g. snapin and PP2A (protein phosphatase 2A)] which bind the N-terminus of different ACs have been reported (3), suggesting that the N-terminus might function as a scaffold which selectively mediates the cross-talk between the cAMP signaling and other machinery. AC5 contains a very long N-terminus (242 amino acids) that interacts with Ric8a as binding partner of the N-terminus of AC5. Ric8a has been demonstrated to associate with many Ga proteins in mammalian cells, including *Gas*, *Gaq*, *Gao*, *Gai* and *Gai3* (4). The association of Ric8A with AC5 N-terminal region suppresses the AC5 activity via a Galfai-dependent pathway. Ric-8A interacts with GDP-bound Galphai proteins, stimulates release of GDP, and forms a stable nucleotide-free transition state complex with the Galphai protein; this complex dissociates upon binding of GTP to Galphai (4). The Ric8A-selective rabbit antibodies were generated against a unique epitope on human Ric8A homolog near C-terminal end of the protein. The synthetic peptide was covalently modified to achieve desired antigenic and was conjugated to a carrier protein before used as immunogen to raise antibodies. The Ric8A antibodies are affinity purified over immobilized immunogenic peptide affinity matrix and stabilized with preservatives for long-term storage. FabGennix provides FITC- and Biotinylated conjugates of this antibody for direct applications in IHC applications. FabGennix Int. Inc. also provides limited quantities of antigenic blocking peptide for immune depletion assays wide range of antibodies to protein kinases (receptor and non-receptor kinases) and kinase inhibitors. *FabGennix Inc.* will also conjugate antibodies with fluorescent probes upon request at a reasonable cost.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Vol
Ric8A-101AP	Rabbit	Affinity purified Ric8A antibodies	Human, mouse	100 ug	200ul
FITC-Ric8A	Rabbit	FITC-Conjugated Ric8A antibody	Human, mouse	250ug	200ul
Biotin-Ric8A	Rabbit	Biotin-labeled Ric8A antibody	Human, mouse	250ug	200ul
P-Ric8A	n/a	Antigenic blocking peptide for Ric8A-101AP	n/a	250ug	100ul
PC-Ric8A	n/a	Western blot positive control for Ric8A	n/a	5 appl.	inquire

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

- Immunogen:** Synthetic peptide corresponding to amino acids 514-530 of Ric8A protein. The peptide was covalently modified before coupling to a carrier protein for immunogen preparation.
- Concentration:** Ric8A-101AP Ig concentration 0.6-0.75 mg/ml in antibody stabilization buffer.
- Applications:** Antibody Ric8A-101A is ideal for IMM/WB applications for detection of endogenous Ric8A protein. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions. Laboratory. Western blotting: > 1:250; IMM: Immunoprecipitation 1:200 recommended; IHC = nd
- Reactivity:** This antibody detects two bands of approximately 68kDa in Ric8A Western Blot positive controls (PC-Ric8A). The antibody also labels some non-specific/unknown proteins at 40kDa range. The identity of these proteins are not known.
- Protocols:** Standard protocol for various applications (WB; IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.
- Form/Storage:** The antiserum is supplied in antibody stabilization buffer with 0.02% thimerosal or merthiolate. The affinity-purified antibodies are purified on antigen-spharose affinity column and supplied as stabilized antibody. For long-term storage of antibodies, store at -20°C. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µm filter after every use for long-term storage.
- References:**



Western blot of Ric8A-101AP using PC-Ric8A sample. Antibody dilution 1:500 in diluOBuffer

- Marc Mirande. The ins and outs of tRNA transport. *EMBO Rep.* 2007 June; 8(6): 547-549.
- Chatterjee S, Home P, Mukherjee S, Mahata B, Goswami S, Dhar G, Adhya S. An RNA-binding respiratory component mediates import of type II tRNAs into *Leishmania* mitochondria. *J. Biol. Chem.* 2006; **281**:25270-25277.
- Tall GG, Krumins AM, Gilman AG. Mammalian Ric-8A (*synembryn*) is a heterotrimeric Galphai protein guanine nucleotide exchange factor. *J Biol Chem.* 2003 Mar 7; **278**(10):8356-62. Epub 2002 Dec 30.

For users who may require large amounts of P65PAK-101AP, please enquire about bulk material discounts.
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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