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Customer Service: 1-800 786 1236
Technical Service: 214 387 8105
Fax: 214 387 0870
Info@fabgennix.com
www.fabgennix.com

Antibodies to C. elegans and other nematodes proteins

Endogenous RNAi deficient arGOnaute family member 1 (ergo-1) antibodies (Cat # Ergo-101AP, P-Ergo and PC-Ergo1)

Alternate nomenclature: Ergo 1, Endogenous-RNAi-deficient Argonaute family member (Ergo-1), hypothetical protein R09A1.1.

In mammalian cells miRNA-induced silencing complexes (miRISC) are formed by microRNAs, which regulate protein expression post transcriptionally through binding to 3'-untranslated regions of the target mRNAs. At least 4 Argonaute proteins (Ergo1-4) have been identified. Recent studies have identified a conserved WG/GW-containing motif, known as the Argonaute (AGO) hook, which is involved in the recruitment of AGOs to distinct components of the eukaryotic RNA silencing pathways. Argonaute proteins are important component of the miRISC complexes. Argonaute 2 (Ergo-2) recruits GW182, a key component of miRISC of the processing body (GW/P-body) to target mRNA. Ergo-2 interacts with GW182 at three binding sites within the amino terminal (GW/GW)-repeat region that is characteristics of the GW182 family proteins. It is been shown that multiple Ergo proteins are connected via GW182 proteins. The interaction of Ergo with GW182 family proteins relieved let-7-mediated repression of protein synthesis in mammalian cell-free system, as a result of delayed let 7-directed target mRNA deadenylation (1). These data suggest that that the interactions of GW182 with Argonautes may induce the formation of large complexes containing miRNA target mRNAs, and may be critical for miRNA-mediated translational repression.

The 97-megabase genomic sequence of the nematode *Caenorhabditis elegans* reveals over 19,000 genes. More than 40 percent of the predicted protein products find significant matches in other organisms. There is a variety of repeated sequences, both local and dispersed. The distinctive distribution of some repeats and highly conserved genes provides evidence for a regional organization of the chromosomes (2). One of the protein sequenced from *Caenorhabditis elegans* was Endogenous-RNAi-deficient Argonaute family member protein 1 (ergo-1). The protein comprise of 112 amino acids, calculated MW of 126.5kDa, with several function conservative domains. This protein is part of the RNA-induced silencing complex (RISC) and is an endonuclease that plays a key role in RNA interference pathways. The PAZ domain (amino acid 462-533) was named after protein containing Piwi, Argonaute and cd02846. The Piwi domain is the c-terminal portion of the Argonaute protein and contains two sub domains. The protein also has a nucleic acid binding interface and a 5'RNA guide strand anchoring site. guide

The Anti-Endogenous-RNAi-deficient Argonaute family (Ergo-1) protein antibodies were generated against a synthetic peptide corresponding to C-terminal location of Ergo 1 protein from *Caenorhabditis elegans*. The Ergo 1-selective antibodies are affinity purified against immobilized antigen based affinity chromatography which yielded high affinity epitope-specific antibodies. The Ergo1 antibodies label a protein of approximately 127kDa Da in Ergo 1 western blot positive control samples (PC-ergo1). Anti-Ergo1-selective antibodies are also available in FITC-conjugated forms (FITC-Ergo1) for confocal, Western blotting and immunocytochemical analyses. *FabGennix Inc.* will also conjugate this and all its other antibodies with fluorescent probes upon request at a nominal extra charge. Limited quantities of antigenic blocking peptide and western blot positive control for Ergo1 are also available (please inquire their availability before ordering). *FabGennix Inc.* has produced and characterized antibodies for epigenetics and, cell cycle-related research areas, for a complete listing of these products, please visit www.FabGennix.com. *FabGennix Int. Inc.*, provides custom antibody development services specially for post-translationally modified epitopes including phosphorylation, lipid modifications, glycosylations, oxidations etc.). Please inquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Volume
Ergo1-101AP	Rabbit	Affinity purified endogenous RNAi deficeint argonaute antibody	C. elegans	200ul	175ul
FITC-Ergo1	Rabbit	Affinity purified FITC-conjugated Ergo1 antibody	C. elegans	200ul	for 5 appl
P-Ergo1	n/a	Antigenic blocking peptide for Ergo1-101AP antibody	n/a	250 mg	100 ul
PC-Ergo1	n/a	Western blot positive control for C elegans Ergo1	n/a	5 appl	inquire

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

Immunogen: Synthetic peptide corresponding to amino acids 1103-1121 of the C. elegans Ergo1 protein with amino acids (cev nkd mnv nek leg mtf v), the peptide was amidated and conjugated to a carrier protein for preparation of antigen to generate antibodies in rabbit.

Concentration: Ergo1-101AP and FITC-Ergo1 affinity purified antibodies are at concentration 0.62-0.68 mg/ml of antibody stabilization buffer.

Applications: Ergo1-101AP is tested for WB application at 1:500 dilution. Other applications for this antibody has not been tested. WB: > 1:500; IMM & i.p pull-down assays: n.d; IHC n.d (Antibody dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions). Investigators who want to use this antibody in applications not listed here can ask for a complimentary sample of Ergo1-101AP antibodies. We will be happy to provide this antibody form multiple rabbits.

Protocols: Standard protocol for various applications (WB, IMM, IHC) of this antibody can be obtained upon request. The specification sheet for Ergo1-101AP antibody will be supplied with each product. *FabGennix Inc.* strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% azide. The purified antibodies are isolated from immobilized antigen affinity column. The affinity pure antibodies are supplied as 0.5-1 mg/ml IgG in antibody stabilization buffer. For long-term storage of keep 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 0.45 filter after every use for long-term storage.

Notes: Now Western blots can easily be stripped and recycle using our specially formulated StripOBuffer (Cat # FGI-1989). This stripping buffer does not require heating or have any pungent smell.

References:

1. Takimoto K, Wakiyama M, Yokoyama S. Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression. *RNA*. 2009 Apr 27. [Epub ahead of print]
2. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *C. elegans* Sequencing Consortium. *Science*. 1998 Dec 11;282(5396):2012-8. Links Erratum in: *Science* 1999 Jan 1;283(5398):35; *Science* 1999 Mar 26;283(5410):2103; *Science* 1999 Sep 3;285(5433):1493.

* For users who may require large amounts of Ergo1-101AP, 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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5850 Town and Country Blvd. Suite 301. Frisco, TX 75034

Customer service: 1800 786 1236; Technical Support: 214 387 8105