

New Item

INTERNATIONAL

ADP-Ribosylation factor 1 (ARF 1) antibodies. Catalog # ARF1-101AP, FITC-ARF1, Biotin-ARF1, P-ARF1 and PC-ARF1.

Accession # and Alternate Nomenclature: P84079,

Ras superfamily of small GTPases regulates a wide variety of cellular processes including intracellular trafficking of proteins. The Ras proteins acts as molecular switches by cycling between inactive GDP-bound and active GTP-bound state. Guanine exchange factors (GEFs) Among the various Ras proteins, the ADP-ribosylation factor proteins (ARFs) are well characterized regulators of vesicle formation in intracellular membrane traffic while Rho proteins regulate the actin cytoskeleton (1). The Arf family members have unique structural device, called the 'interswitch toggle', that implements communication between the nucleotide-binding site and a unique N-terminal amphipathic helix that mediates the interaction of Arf proteins with membranes, these structural motifs distinguish them from other small GTPases (2). When GDP is bound to ARF proteins the amphipathic helix is positioned in a hydrophobic pocket, whereas in the GTP-bound form it is displaced by the interswitch toggle and stabilizes interaction of the protein with membrane lipid bilayer. Thus, the GTP-GDP cycle is coupled to membrane-cytosol cycle and thereby ARF proteins are able to regulate vesicular traffic and organelle structure formation (2).

The mammalian ARF family comprise of 5 members (ARF1-6), there is no ARF2. ARF1 and ARF6 are most abundantly expressed and located in distinct cellular locations at Golgi complex and early-endosome-plasma membrane interface and are most distantly related with only 67% sequence identity. ARF1 is mostly known for its role in vesicle budding and Golgi regulation through the recruitment of coat proteins and in the regulation of lipid-modifying enzymes and actin organizing components (3). The crystal structure of ARF-BD (ARF binding domain) comprise of a PH domain adjacent to a C-terminal helix and ARF interacts with these two regions via its "interswitch toggle" (4). ARF1 protein has 188 amino acids and is expressed in at least 3 variant forms, ARF1 antibodies will label all variants equally. The deactivation of ARF1 induces redistribution of entire Golgi complex to ER, the GTP bound form triggers the association with coat protein with the Golgi membranes. Purified ARF proteins do not have GTPase activity suggesting that GTP hydrolysis is dependent on GTPase activating protein (GAP) a 49kDa protein (4). The GTP bound ARF1 and 3 proteins also interact with several interacting protein (ARFGAPs) including protein interacting with C kinase 1 (PICK1) to mediate their cellular processes.

ARF1-selective antibodies were generated against unique antigenic peptide sequences form ARF1 protein, this peptide sequence was not found in any other protein listed in the gene bank. The ARF1 antibodies were affinity purified over immobilized antigen based chromatography, and the purified immunoglobulins are stabilized in antibody stabilization buffer. FabGennix Int. Inc., will also provide limited quantities of antigenic blocking peptide for ARF1. Antibodies to several other Ras targets are available from FabGennix International Inc.

Inc. will conjugate antibodies with secondary enzymes (alk-Pase or HRP) or fluorescent probes upon request at a nominal cost. FabGennix Int. Inc., will also provide western blot positive controls for it antibodies in ready-to-use buffer. Limited quantities of antigenic blocking peptide is available (Please inquire before orders).

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Volume
ARF1-101AP	Rabbit	Affinity purified ARF1-selective antibodies	R, M, H	100 ug	200ul
ARF-FITC	Rabbit	FITC-conjugated ARF1 antibodies	R, M, H	100ug	200ul
Biotin-ARF1	Rabbit	Biotin-labeled ARF1 antibodies	R, M, H	100ug	200ul
P-ARF1	n/a	Antigenic blocking peptide for ARF-101AP	n/a	250 ug	100ul
PC-ARF1	n/a	Western blotting positive control for ARF1	n/a	For 5 appl	inquire

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

Immunogen: Synthetic peptides corresponding to unique epitope on ARF1 protein. The peptide sequence was unique to ARF1 protein and was not present on any other ARF family members. The ARF1 peptide was covalently modified post-synthetically covalently modified to achieve desired antigenicity.

Concentration: ARF1-101AP: IgG concentration 0.64-0.72 mg/ml in antibody stabilization buffer.

Applications: Antibody ARF1-101AP is ideal for WB and ELISA applications, other applications have not been tested. These antibodies do not cross react to other members of the Ras family or to other ARF members. The species cross reactivity for these antibodies have not been examined fully. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay. WB: > 1:500; IMM & i.p pull-down assays: n.d; IHC n.d. ELISA <1:10,000. Application of this antibody in protocols not listed here does not necessarily exclude its use in such procedures.

Reactivity: This antibody detects a single band of approximately 20kDa in PC-ARF1 samples.

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. The affinity-purified antibodies are isolated on immobilized antigen-affinity column and supplied as stabilized product. Store at -20°C for long-term storage. 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 0.45um filter after every use for long-term storage.

Notes: Briefly centrifuge to collect liquid, heat or boil PC-ARF1 tube for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature, Repeated freezing and thawing may result in appearance of higher MW immunoreactive bands.

New Reagents: Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating required. Block in 5X diluOBuffer and you are ready for blotting with a new antibody

References:

- Hall A (2005) Rho GTPases and the control of cell behavior. *Biochem Soc Trans* 33: 891-895
- Antony B, Beraud-Dufour S, Chardin P, Chabre M (1997) *Biochemistry* 36: 4675-4684
- D'Souza-Schorey C, Chavrier P (2006) ARF proteins: roles in membrane traffic and beyond. *Nat Rev Mol Cell Biol* 7: 347-358
- Julie Ménétrey, Mylène Perderiset, Jérôme Cicolari, Thierry Dubois, Nadia Elkhatib, Fatima El Khadali, Michel Franco, Philippe Chavrier, and Anne Houdusse. *EMBO J* .26(7); Apr 4, 2007
- Makler V, Cukierman E, Rotman M, Admon A, Cassel D. ADP-ribosylation factor-directed GTPase-activating protein. Purification and partial characterization. *J Biol Chem*. 1995 Mar 10;270(10):5232-7. Links

* For users who may require large amounts of ARF1-101AP, 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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