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INTERNATIONAL

New Item New Item

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Non-Receptor Tyrosine kinases selective antibodies

Anti-ACK1 antibodies (ACK-100P, ACK-101AP)

ACK1 (activated Cdc42-associated kinase 1) is a nonreceptor tyrosine kinase and the only tyrosine kinase known to interact with Cdc42. ACK1 phosphorylates and activates the guanine nucleotide exchange factor Dbl, which in turn directs the Rho family GTP-binding proteins (1). ACK1 interacts most strongly with the SH3 domains of Src family kinases (Src or Hck) via its C-terminal proline-rich domain. The ACK1 interacts most strongly with the SH3 domains of Src family kinases (Src or Hck) by its C-terminal proline rich domain. ACK1 also acts as a regulator of Dbl, which in turn activates Rho family proteins. There are at least 2 ACK1 associated proteins (Cdc42 and Grb) that are required for ACK1-dependent EGF signaling in the cell. The central motif in ACK1 has a clathrin adaptor that colocalizes ACK1 with in the clathrin containing vesicles that are involved in the trafficking of receptor mediated endocytosis (2).

The ACK family of non-receptor tyrosine kinase (ACK1 and ACK2) specifically associate with Cdc42 to modulate its activity in several signaling pathways. ACK1 acts as a mediator of EGF signal to Rho family GTP-binding proteins (3). The activation of ACK1 involves auto-phosphorylation at Tyrosine 284, upon phosphorylation, the ACK1 activity is significantly enhanced. Tyrosine substitution at 284 by Phenylalanine (Y284F) and expression in COS-7 cells exhibit dramatically reduced levels of tyrosine phosphorylation (1). ACK2 mediates cell adhesion signals initiated by integrins $\beta 1$ in a Cdc-dependent manner (4). There are three structural domains associated with ACK1 protein, a N-terminal tyrosine kinase domain, SH3 domain, followed by a Cdc42/Rac interactive binding (CRIB) domain and a proline rich region near C-terminal end. ACK2 has been shown to be activated by Cdc42 binding to the CRIB region (4).

The ACK1 protein is a approximately 118-120kDa (1036 amino acids) protein. The Anti-ACK1-selective antibody was generated against a peptide from the tyrosine kinase domain that is unique to ACK1 protein. The affinity purified mono-specific polyclonal antibody strongly labels a 118-120kDa protein in ACK1 western blot positive controls. *FabGennix Inc.* will also conjugate antibodies with fluorescent probes upon request at extra charge. *FabGennix Inc.* also provides antibodies against proteins that are involved in ACK1 signalling including ACK2, phosphor-ACK1, Herceptins (Erbs) and others. *FabGennix Inc.* employs cyclic peptide methodology for generating antibodies, which results in higher titer and specificity (6). *FabGennix, Inc.*, will also provide Western blot positive controls for phosphor and non-phospho ACK1 in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigens are also available. Please enquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Price
ACK-100P	Rabbit	Polyclonal antisera	R, M, H, monk	100 μ l	205
ACK-101AP	Rabbit	Affinity purified IgG	R, M, H, monk	100 μ g	235
P-ACK1	Rabbit	Antigenic blocking peptide	R, M, H, monk	250 μ g	165
PC-ACK1	cells	Western blot positive control for ACK1	R, M, H, monk	5 appl	270
PC-PACK1	cells	Western blot positive control for phospho-ACK1	R, M, H, monk	5 appl	235

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

Immunogen: Synthetic peptide unique to ACK1 protein

Concentration: ACK-101AP IgG concentration 0.65-1.25 mg/ml.

Applications: Antibody ACK-101AP is ideal for IMM and WB. ACK-101AP 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 μ l/250 μ g protein extracts)

Reactivity: This antibody detects a single 100kDa ACK1 protein in PC-ACK1 samples. The antibody also reacts to a 100 kDa protein in various breast cancer cell lines.

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

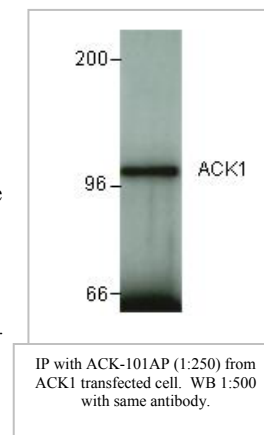
Form/Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide. For long-term storage of antibodies, store at -20°C. *FabGennix Int. Inc.* does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody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

References:

1. Kato-Stankiewicz J, Ueda S, Kataoka T, Kaziro Y, Satoh T. *Biochem Biophys Res Commun.* 2001 8;284:470-7)
2. Teo M, Tan L, Lim L, Manser E. *J Biol Chem.* 2001 May 25;276(21):18392-8. Epub 2001 Feb 27
3. Kato-Stankiewicz et al., *Biochem. Biophys. Res. Comm.* 284, 470-477, 2004.
4. Yang W., et. Al., *J. Biol. Chem.* 274, 8524-8530, 1999.

Announcements: A new Phospho-Specific ACK1 Antibody (Cat # PACK-140AP) is now available from *FabGennix International Inc.* This antibody is raised against a synthetic peptide corresponding to autophosphorylation site on ACK1 protein (1).

*For users who may require large amounts of MERTK-100P or MERTK-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.



011502-0020SF1001Z-rev10.00

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