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### Antibodies to Protein Phosphatase Slingshot 1 isoform 1 (SSH1)

Slingshot 1 isoform 1 antibodies. Catalog # SISHT-101AP, P-SISHT and PC-SISHT.

Accession # NP\_061857 and Alternate Nomenclature: *Slingshot homolog 1; SSH1L; FLJ21928; FLJ38102; KIAA1298*

Slingshot 1 is a member of slingshot (SSH) family of protein phosphatases; it is located at human chromosome 12q24.11. Slingshot is one of the primary dedicated cofilin phosphatases. It plays an important role in cell migration by dephosphorylating cofilin at Ser3 and activating it in a variety of cell types such as keratinocytes and aortic smooth muscle cells (1). Slingshot (SSH) activity can be modulated by F-actin binding, calcium acting via calcinerium, and by its association in inhibitory complexes with 14-3-3 regulatory proteins (2). 14-3-3zeta/tau heterodimers are key regulators of slingshot 1 isoform 1 (SSH1) activity in keratinocytes (3). SSH1 is involved in H<sub>2</sub>O<sub>2</sub> induced cofilin dephosphorylation and activation. Such activation of SSH1-cofilin pathway stimulates the SSH1 dependent formation of cofilin-actin rods in HeLa cells (4). By dephosphorylating and activating cofilin, SSH1 plays a critical role in insulin-induced membrane protrusion and cytokinesis.

SSH1 has the SSH family-specific, N-terminal, noncatalytic (SSH-N) domain. This domain has been shown to play critical roles in P-cofilin recognition, F-actin-mediated activation, and subcellular localization of SSH1 (5). Recently it was shown that expression of phosphatase-dead versions of SSH proteins resulted in phosphorylation/inactivation of cofilin, changes in cytoskeleton organization, loss of cell polarity, and assembly of aberrant arrays of laminin-332 in human keratinocytes (6). SSH1 mutations were seen in patients with disseminated superficial actinic porokeratosis. SSH1 is expressed in a various cell types including keratinocytes, HeLa cells and smooth muscle cells. It is approximately a 126kDa protein (1049 amino acids).

The SSH1-selective antibodies were generated against purified Slingshot1 isoform1 (SSH1) protein. SSH1-antibodies are 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 protein for SISHT -101AP antibody. Antibodies to SSH1 (SISHT-101AP) will label ~126kDa protein in Western blot positive control samples for SSH1 and several other tissues. FabGennix Inc. will also conjugate antibodies with secondary enzymes (alk-Pase or HRP) or fluorescent probes upon request at nominal cost.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Volume
SISHT-101 AP	Rabbit	Affinity purified SISHT antibodies	n.d	100 ug	200ul
FITC- SISHT	Rabbit	FITC-conjugated SISHT antibody	n.d	100ug	200ul
P- SISHT	n/a	Antigenic blocking peptide for SISHT -101AP	n/a	250 ug	100ul
PC- SISHT	n/a	Western blotting positive control for SISHT	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 SISHT. The SISHT peptide was covalently modified post-synthetically covalently modified to achieve desired antigenicity.

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

**Applications:** Antibody SISHT -101AP is ideal for WB and ELISA applications, other applications have not been tested. 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 126kDa in PC- SISHT 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-SISHT 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:**

1. Wang Y, Shibasaki F, Mizuno k. Calcium signal-induced cofilin dephosphorylation is mediated by slingshot via calcinerium. J Biol Chem. 2005; 280:12683-12689.
2. Huang TY, DerMardirossian C, Bokoch GM. Cofilin phosphatases and regulation of actin dynamics. Curr Opin Cell Biol. February 2006; 18(1):26-31.
3. Kligys K, Yao J, Yu D, Jones JC. 14-3-3zeta/tau heterodimers regulate slingshot activity in migrating keratinocytes. Biochem Biophys Res Commun. 12 June 2009; 383(4): 450-454.
4. Kim J-S, Huang TY, Bokoch GM. Reactive oxygen species regulate a slingshot-cofilin activation pathway. Mol Biol Cell. 1 June 2009; 20(11):2650-2660.
5. Kurita S, Watanabe Y, Gunji E, Ohashi K, Mizuno K. Molecular dissection of the mechanisms of substrate recognition and F-actin-mediated activation of cofilin-phosphatase slingshot-1. J Biol Chem. 21 November 2008; 283(47):32542-32552.
6. Kligys K, et. al. The slingshot family of phosphatases mediates Rac1 regulation of cofilin phosphorylation, laminin-332 organization, and motility behavior of keratinocytes. J Biol Chem. 2 November 2007; 282(44):32520-32528.

\* For users who may require large amounts of SISHT-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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