

#2725 Store at -20°C

# SHIP1 (C15C9) Rabbit mAb

100 µl  
 (10 Western mini-blot)



**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
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New 10/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W, IP, F	H	145 kDa	Rabbit**	IgG

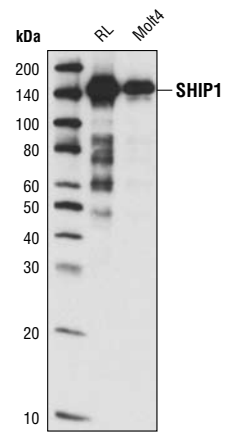
**Background:** SH2-containing inositol phosphatase 1 (SHIP1) is a hematopoietic phosphatase that hydrolyzes phosphatidylinositol-3,4,5-triphosphate to phosphatidylinositol-3,4-bisphosphate (1). SHIP1 is a cytosolic phosphatase with an SH2 domain in its amino terminus and two NPXY Shc binding motifs in its carboxy terminus (1,2). Upon receptor cross-linking, SHIP is first recruited to the membrane junction through binding of its SH2 domain to the phospho-tyrosine in the ITIM motif (2), followed by tyrosine phosphorylation on the NPXY motif (2). The membrane relocalization and phosphorylation on the NPXY motif is essential for the regulatory function of SHIP1 (3-5). Its effect on calcium flux, cell survival, growth, cell cycle arrest and apoptosis is mediated through the PI3K and Akt pathways (3-5). Tyrosine 1021 is located in one of the NPXY motifs in SHIP1, and its phosphorylation is important for SHIP1 function (6).

**Specificity/Sensitivity:** SHIP1 (C15C9) Rabbit mAb detects endogenous levels of total SHIP1 protein.

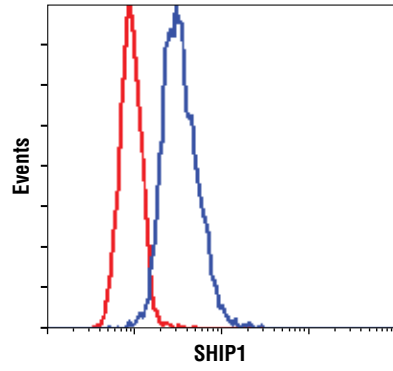
**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (Blue Carrier-coupled) corresponding to residues surrounding Pro1135 of human SHIP1.

**Background References:**

- Tridandapani, S. et al. (1997) *Mol. Cell Biol.* 8, 4305-4311.
- Liu, L. et al. (1997) *J. Biol. Chem.* 272, 8983-8988.
- Malbec, O. et al. (2001) *J. Biol. Chem.* 276, 30381-30391.
- Carver, D.J. et al. (2000) *Blood* 96, 1449-1456.
- Scharenberg, A.M. et al. (1998) *EMBO J.* 17, 1961-1972.
- Sattler, M. et al. (2001) *J. Biol. Chem.* 276, 2451-2455.



Western blot analysis of extracts from RL and Molt4 cells using SHIP1 (C15C9) Rabbit mAb.



Flow cytometric analysis of Jurkat cells (red) and RL cells (blue) using SHIP1 (C15C9) Rabbit mAb.

**Entrez-Gene ID #** 3635  
**Swiss-Prot Acc. #** Q92835

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by Western blot.**

**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:50
Flow Cytometry	1:200

**Companion Products:**

- Phospho-SHIP1 (Tyr1020) Antibody #3941
- SHIP1 (P290) Antibody #2726
- SHIP1 (D1163) Antibody #2728
- Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058
- Akt Antibody #9272
- PI3 Kinase p85 Antibody #4292
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.

## Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g/ml}$  Leupeptin

**NOTE:** Add 1 mM PMSF immediately prior to use.

- Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
- Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
- 3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

### B Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
- Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
- Sonicate samples on ice three times for 5 seconds each.
- Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

### C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

- Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
- Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
- Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
- Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
- Analyze sample by Western blotting (see Western Immunoblotting Protocol).

### Cell Lysate Pre-Clearing (Optional)

- Take 200  $\mu\text{l}$  cell lysate and add to either Protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry).
- Incubate at 4°C for 30 – 60 minutes.
- Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
- Proceed to step 1 of Immunoprecipitation.

## Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

### A Solutions and Reagents

- 1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g  $\text{Na}_2\text{HPO}_4$  and 0.24 g  $\text{KH}_2\text{PO}_4$  in 800 mL distilled water ( $\text{dH}_2\text{O}$ ). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
- Formaldehyde (methanol free)
- Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

### B Fixation

- Collect cells by centrifugation and aspirate supernatant.
- Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
- Fix for 10 minutes at 37°C.
- Chill tubes on ice for 1 minute.

### C Permeabilization

- Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
- Incubate 30 minutes on ice.
- Proceed with staining or store cells at -20°C in 90% methanol.

### D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

**NOTE:** Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

- Aliquot 0.5-1x10<sup>6</sup> cells into each assay tube (by volume).
- Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
- Resuspend cells in 100  $\mu\text{l}$  Incubation Buffer per assay tube.
- Block in Incubation Buffer for 10 minutes at room temperature.
- Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
- Incubate for 30-60 minutes at room temperature.
- Rinse as before in Incubation Buffer by centrifugation.
- Resuspend cells in fluorochrome-conjugated secondary antibody\*, diluted in Incubation Buffer according to the manufacturer's recommendations.
- Incubate for 30 minutes at room temperature.
- Rinse as before in Incubation Buffer by centrifugation.
- Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

\*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')<sub>2</sub> fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)  
A-11017 Alexa Fluor® 488 F(ab')<sub>2</sub> fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)