Monoclonal Anti-human/mouse/rat SHIP Antibody

ORDERING INFORMATION

Catalog Number: MAB2317

Clone: 257812

Lot Number: KXV02

Size: 100 μg (sufficient for 100 mL of blotting solution)

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

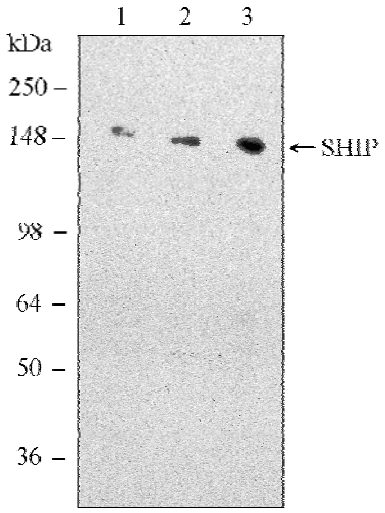
Storage: -20° C

Specificity: human, mouse, and rat SHIP

Immunogen: *E. coli*-derived rmSHIP

Ig class: rat IgG_{2A}

Recommended Applications:
Western blot
Immunohistochemistry



Detection of SHIP with MAB2317. 10 μg, 20 μg, and 40 μg of TF-1 cell lysates (lanes 1, 2, and 3, respectively) were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1 μg/mL MAB2317, as described in *Protocols for Immunoblotting*. A 1 minute exposure to film is shown.

Background
SH2-containing inositol phosphatase (SHIP), also known as INPP5D, is a negative regulator of signal transduction in hematopoietic cells. Targeted disruption of SHIP in mice leads to a myeloproliferative disorder. Several laboratories have demonstrated the presence of multiple forms of SHIP, including 145 kDa, 135 kDa, and C-terminal truncated forms at 125 kDa and 110 kDa in some cell types.

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived recombinant mouse SHIP (rmSHIP; aa 874 - 941; Accession # U39203). The IgG fraction of the tissue culture supernatant was purified by protein G chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution
Reconstitute in PBS containing 0.02% NaN_3.

Storage
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody detects endogenous human, mouse, and rat SHIP proteins of 145, 135, 125, and 110 kDa using Western blots.

Applications
Western blot - An antibody concentration of 1.0 μg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	2% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1 μg/mL anti-human/mouse/rat SHIP.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated goat anti-rat IgG (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell Lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Immunohistochemistry - A biotin conjugate of this antibody was used at a concentration of 25 μg/mL with appropriate secondary reagents to detect SHIP in mouse splenocytes. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by the individual laboratory.

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