ANTIBODIES

Product Information Sheet

ANTI-PHOSPHOSHIP

Affinity Purified Rabbit Polyclonal Antibody

Catalog #01507 0.1 mL

SCIENTIFIC BACKGROUND:
SHIP (SH2 containing inositol 5' phosphatase) is a hematopoietic-restricted 145 kDa protein that becomes tyrosine phosphorylated following cytokine, growth factor, chemokine, and immuno-receptor stimulation. SHIP hydrolyzes the critical phosphatidylinositol (PI)-3-kinase (PI3K)-generated second messenger, PI-3,4,5-P_3 (PIP_3), to PI-3,4-P_2 [1,2] and therefore acts as an important negative regulator of the PI3K pathway.

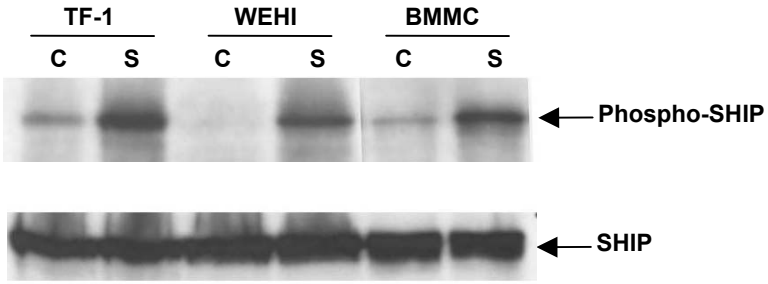
SPECIFICITY:
This antibody reacts with mouse and human tyrosine phosphorylated SHIP.

IMMUNOGEN:
This polyclonal antibody was generated against a phosphopeptide corresponding to residues surrounding the phosphorylated tyrosine pY^{1020} in human SHIP.

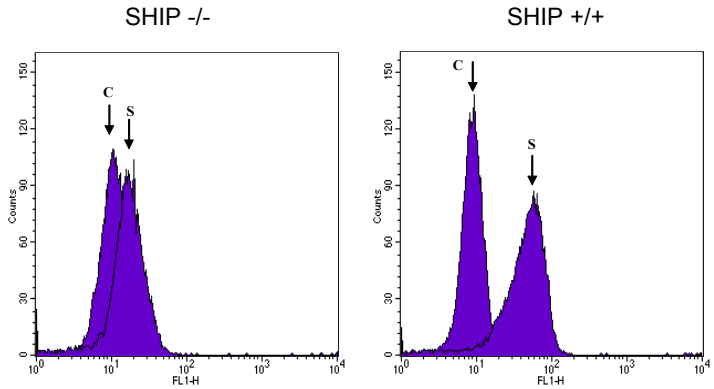
FORMAT:
0.1 mL of immunogen-affintiy purified rabbit polyclonal antibody in 0.01% BSA, 0.05% sodium azide and 50% glycerol (pH 7.4). Liquid at -20°C.

STABILITY AND STORAGE:
Anti-phosphoSHIP can be stored at -20ºC to -70ºC for at least 1 year without detectable loss of activity.

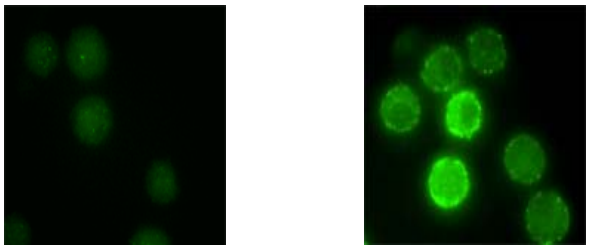
IMMUNOBLOT ANALYSIS:
Representative Western Blot of total cell lysates (3 x 10^5 cells) from mouse WEHI-231 cells stimulated with anti-IgM, human TF-1 cells stimulated with IL-3 or mouse bone marrow derived mast cells (BMMCs) stimulated with Stem Cell Factor (SCF). Lysates were resolved by electrophoresis, transferred to a PDVF membrane (Immobilon – Millipore, Nepean, ON) and probed with anti-phosphoSHIP (1:5000 dilution, C refers to unstimulated and S to stimulated samples). Proteins were visualized using goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (HRP) and a chemiluminescence detection system. Arrow indicates phosphoSHIP (145 kDa). The blot was re-probed with anti-SHIP (1:1000, P1C1, Santa Cruz) to show equal loading of C and S samples.



IMMUNOFLUORESCENCE ANALYSIS:
FACS:
Bone marrow derived mast cells from normal or SHIP knockout mice either unstimulated (C-control) or stimulated (S) for 2 minutes with 200 ng/mL SCF, fixed, permeabilized (FIX&PERM – Caltag, Burlingame, CA) and labelled with anti-phospho SHIP (1 in 500 dilution) followed by anti-rabbit AlexaFluor488 (Molecular Probes, Eugene, OR, 1 in 200 dilution).



Immunofluorescence Microscopy:



C-control S

Bone marrow derived mast cells either unstimulated (C-control) or stimulated (S) for 2 minutes with 200 ng/mL SCF were fixed, permeabilized (FIX&PERM – Caltag, Burlingame, CA) and labelled with anti-phospho SHIP (1 in 500 dilution) followed by anti-rabbit AlexaFluor488. BMMCs were allowed to adhere to 0.01% poly-L-lysine coated microscope coverslips and then observed under oil immersion (63X) with an AxioPlan2 fluorescence microscope. Cells labelled with anti-rabbit AlexaFluor488 alone did not show appreciable immunofluorescence (data not shown).

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APPLICATIONS AND DIRECTIONS FOR USE:

Centrifuge tube briefly before use to ensure recovery of entire contents.

Western Blot:
This antibody can be used at a 1 in 2500 dilution with the appropriate secondary reagents to detect phosphoSHIP.

Immunoprecipitation:
Use 5 µL of this antibody per 500 µl of cell lysate from 10^6 mouse WEHI 231 cells. Incubate for 1 hour at 4°C before adding 10 µL of a 50% slurry of immobilized Protein A to isolate phosphoSHIP protein from the cell lysates.

Immunofluorescence:
This antibody can be used at a 1 in 100 to 1 in 1000 dilution with the appropriate secondary reagents to detect phosphoSHIP.

REFERENCE:

1. Damen, JE *et al*, Proc Natl Acad Sci. 93(4):1689-93, 1996.

2. Sly, LM *et al*, Exp Hematol. 31(12):1170-81, 2003.

THIS REAGENT IS FOR RESEARCH USE ONLY.
IT IS NOT TO BE ADMINISTERED TO HUMANS.

Hazardous Ingredient: Sodium Azide. *Avoid exposure to skin and eyes, ingestion, and contact with heat, acids and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharging into plumbing.*

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