

Basic Information

Product Name	Anti-SPHK1 Antibody	
Gene Name	SPHK1	
Source	Rabbit	
Clonality	Polyclonal	
Isotype	IgG	
Species Reactivity	human	
Tested Application	WB, FCM, ELISA	
Contents	500 ug/ml antibody with PBS, 0.02% NaN ₃ , 1 mg/ml BSA and 50% glycerol.	
Immunogen	E.coli-derived human SPHK1 recombinant protein (Position: E55-N357).	
Concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	45-50 kDa	
Dilution Ratios	Western blot (WB):	1:500-2000
	Flow Cytometry (Fixed):	1:50-200
	Enzyme linked immunosorbent assay (ELISA):	1:100-1000

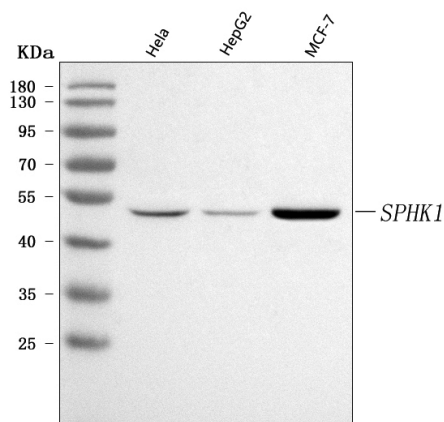
Storage

12 months from date of receipt, -20°C as supplied.

Background Information

SPHK1(Sphingosine Kinase 1), is an enzyme that in humans is encoded by the SPHK1 gene. Melendez et al.(2000) mapped the SPHK1 gene to chromosome 17q25.2 based on sequence identity with ESTs mapped to this region. Kohama et al.(1998) demonstrated that recombinant mouse Sphk1 can specifically phosphorylate D-erythro-sphingosine and that D, L-threo-dihydrosphingosine and N, N-dimethylsphingosine can act as competitive inhibitors of recombinant Sphk1. Pitson et al.(2000) found that recombinant SPHK1 and endogenous SPHK1 purified from placenta had identical enzymatic characteristics, suggesting posttranslational modification does not effect functional properties.

Selected Validation Data



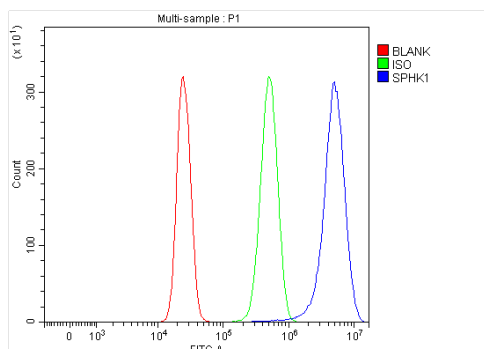
Western blot analysis of anti-SPHK1 antibody (A01390-2). The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human HeLa whole cell lysates,

Lane 2: human HepG2 whole cell lysates,

Lane 3: human MCF-7 whole cell lysates.

After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-SPHK1 antigen affinity purified polyclonal antibody (A01390-2) and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1197). A specific band was detected for SPHK1 at approximately 52 kDa. The expected band size for SPHK1 is at 42 kDa.



Flow Cytometry analysis of HepG2 cells using anti-SPHK1 antibody (A01390-2).

Overlay histogram showing HepG2 cells stained with A01390-2 (Blue line).

To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-SPHK1 Antibody (A01390-2) at 1:100 dilution for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG at 1:100 dilution used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.