

## Anti-SPHK1 Antibody Picoband®

Catalog Number: A01390-1

### About SPHK1

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.

### Overview

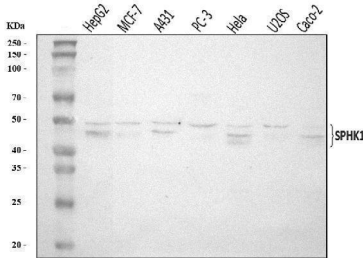
Product Name	Anti-SPHK1 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SPHK1 Antibody Picoband® catalog # A01390-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q9NYA1

### Technical Details

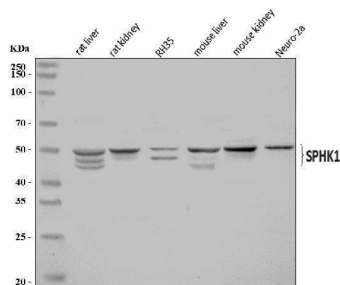
Immunogen	E.coli-derived human SPHK1 recombinant protein (Position: R16-H267).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 ug/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5 ug/ml, -

## Anti-SPHK1 Antibody Picoband® (A01390-1) Images

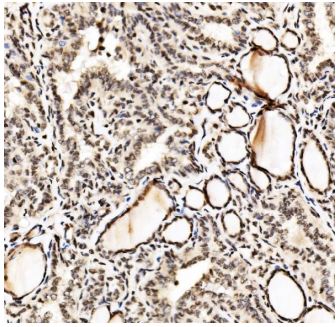


Western blot analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, Lane 2: human MCF-7 whole cell lysates, Lane 3: human A431 whole cell lysates, Lane 4: human PC-3 whole cell lysates, Lane 5: human HeLa whole cell lysates, Lane 6: human U2OS whole cell lysates, Lane 7: human Caco-2 whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-SPHK1 antigen affinity purified polyclonal antibody (Catalog # A01390-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for SPHK1 at approximately 45-50 kDa. The expected band size for SPHK1 is at 45-50 kDa.

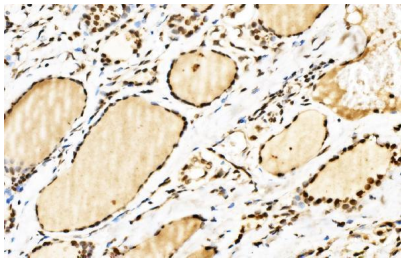


Western blot analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: rat liver tissue lysates, Lane 2: rat kidney tissue lysates, Lane 3: rat RH35 whole cell lysates, Lane 4: mouse liver tissue lysates, Lane 5: mouse kidney tissue lysates, Lane 6: mouse Neuro-2a whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-SPHK1 antigen affinity purified polyclonal antibody (Catalog # A01390-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for SPHK1 at approximately 45-50 kDa. The expected band size for SPHK1 is at 45-50 kDa.

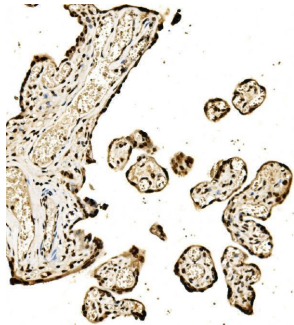
IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human hashimoto's thyroiditis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then



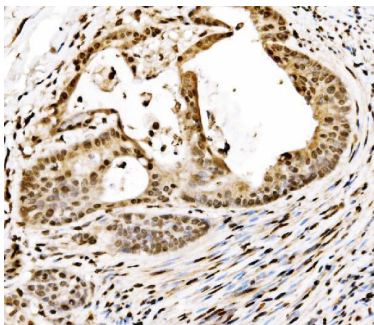
incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human papillary thyroid carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

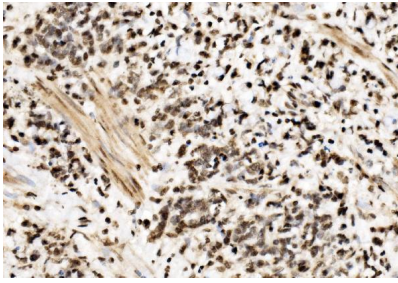


IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

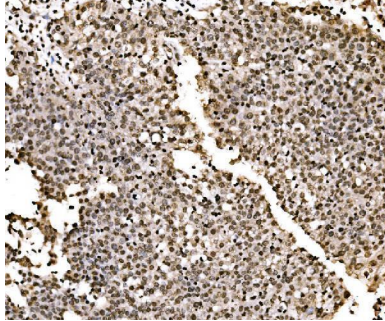


IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human gall bladder adenosquamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

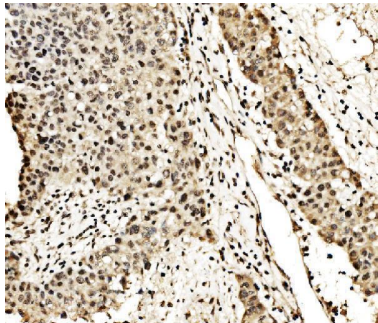
IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated



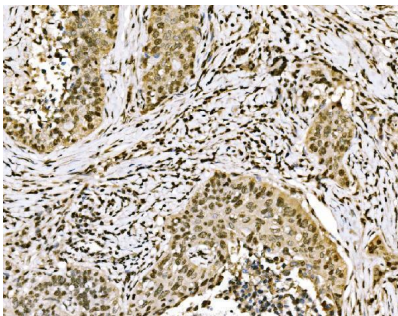
with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human laryngeal squamous cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

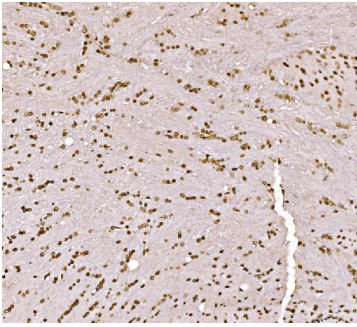


IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

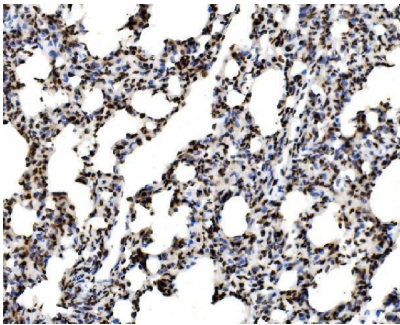


IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

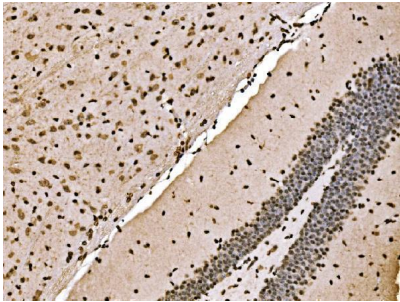
IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary



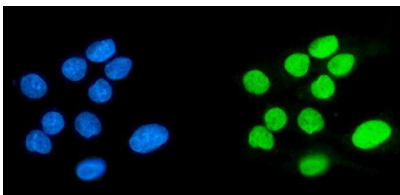
antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

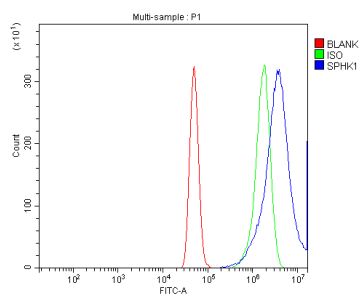


IHC analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IF analysis of SPHK1 using anti-SPHK1 antibody (A01390-1). SPHK1 was detected in an immunocytochemical section of HepG2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-SPHK1 Antibody (A01390-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Flow Cytometry analysis of A431 cells using anti-SPHK1 antibody (A01390-1). Overlay histogram showing A431 cells stained with A01390-1 (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 (A01390-1, 1 ug/1x10<sup>6</sup>



cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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### Anti-SPHK1 Antibody

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