

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human SPHK1 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human SPHK2 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human Sphingosine Kinase 1/SPHK1 Asp2-Leu398 Accession # NP_068807
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

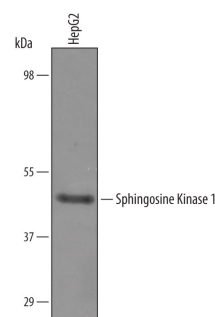
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Immunoprecipitation	25 µg/mL	Conditioned cell culture medium spiked with Recombinant Human SPHK1 (Catalog # 5536-SK), see our available Western blot detection antibodies

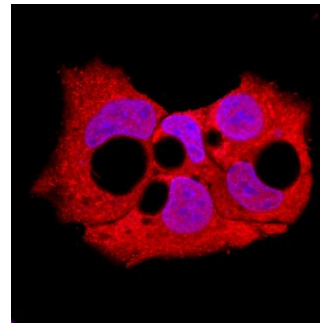
DATA

Western Blot



Detection of Human Sphingosine Kinase 1/SPHK1 by Western Blot. Western blot shows lysates of HepG2 human hepatocellular carcinoma cell line. PVDF Membrane was probed with 0.5 µg/mL of Sheep Anti-Human Sphingosine Kinase 1/SPHK1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5536) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Sphingosine Kinase 1/SPHK1 at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 8](#).

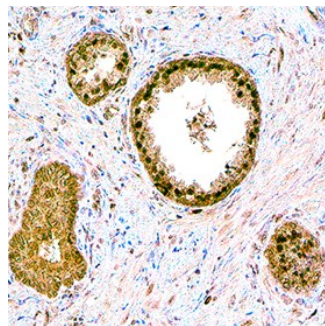
Immunocytochemistry



Sphingosine Kinase 1/SPHK1 in HepG2 Human Cell Line.

Sphingosine Kinase 1/SPHK1 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Sheep Anti-Human Sphingosine Kinase 1/SPHK1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5536) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Sphingosine Kinase 1/SPHK1 in Human Prostate. Sphingosine Kinase 1/SPHK1 was detected in immersion fixed paraffin-embedded sections of human prostate using Sheep Anti-Human Sphingosine Kinase 1/SPHK1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5536) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and nuclei of epithelial cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Sphingosine kinases are cytosolic or membrane-associated enzymes that catalyze the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P). Two types of sphingosine kinases, SPHK1 and SPHK2, are known to be expressed in human cells. The two enzymes share considerable amino acid sequence similarity, but differ in their N-terminal and central regions (1). The two proteins also differ in tissue distribution and some kinetic properties (1). S1P is a lipid messenger that regulates diverse physiological processes including cell proliferation, migration, apoptosis, inflammation, calcium homeostasis and cytoskeletal structure (2, 3). The level of S1P is tightly controlled by SPHKs and S1P degrading enzymes. SPHK1 and its activation can be stimulated by several growth factors such as tumor necrosis factor- α , epidermal growth factor and transforming growth factor- β (3, 4). Expression of SPHK1 has been found to increase in many human solid tumors and overexpression of SPHK1 is associated with tumor angiogenesis (5). Such studies have implicated SPHK1 as a new target for cancer treatment.

References:

1. Liu, H. *et al.* (2000) *J. Biol. Chem.* **275**:19513.
2. Spiegel, S. (1999) *J. Leukocyte Biol.* **65**:341.
3. Alemany, R. *et al.* (2007) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **374**:413.
4. Pederson, L. *et al.* (2008) *Proc. Natl. Acad. Scis USA.* **105**:20764.
5. Shida, D. *et al.* (2008) *Curr. Drug Targets.* **9**:662.