

Product Information



Sphingosine Kinase 1 Polyclonal Antibody

Item No. 10006822 • Lot No. XXXX

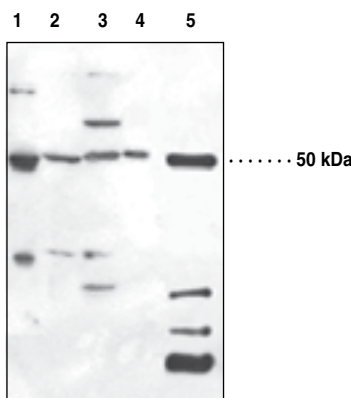
- Contents:** This vial contains *lot specific* µg lyophilized IgG to be reconstituted in 500 µl water. Buffer after reconstitution -1x TBS, pH 7.4.
- Synonym:** SPHK 1
- Antigen:** Human SPHK 1 amino acids 264-274; the antigen alignment with other known sequences is as follows:
- | | |
|-------|-----------------------|
| Human | D L E S E K Y R R L G |
| Mouse | D L E S E K Y R R L G |
| Rat | D L E S E K Y R s L G |
| Frog | D i E S E K Y R f m G |
- Host:** Rabbit
- Cross Reactivity:** (+) Human and mouse SPHK 1, expected to react with rat SPHK 1; other species not tested.
- Stability:** ≥1 year at -20°C
- Applications:** The recommended starting dilution for western blot is (*lot specific* µg/ml) and 1:80 (5 µg/ml) for immunocytochemistry. Other applications were not attempted and therefore optimal working dilutions should be determined empirically.

Sphingosine kinase 1 (SPHK 1) catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate. This reaction plays an important role in determining cell proliferation *versus* cell death.^{1,2} SPHK 1 is found in a wide variety of tissues and cell types including kidney, liver, spleen, heart, platelets, and human tumors. On a cellular level, it is found in the cytosolic and membrane fractions.³ Based on the amino acid sequence, this protein has a molecular weight of approximately 43 kDa. The observed band at 50 kDa may be explained by some reported post translational modifications.⁴

Laboratory Procedures

Immunofluorescent staining of cultured cells

1. Grow cells in multi-well plates as desired.
2. Decant media and wash (attached cells) briefly with TBS, pH 7.4.
3. Fix the cells with 1% formaldehyde in TBS, pH 7.4, for 10 minutes.
4. Wash the cells 3 times with TBS containing 0.1% Triton-X 100 (TBSTX), 10 minutes each.
5. Incubate the cells with 10% normal serum (from the same species in which the secondary antibody is raised) in TBSTX for 30 minutes.
6. Incubate the cells with the SPHK 1 polyclonal antibody (recommended starting concentration of 5 µg/ml. The optimal working condition should be determined by titration) for 1 hour.
7. Wash the cells 3 times with TBSTX, 10 minutes each.
8. Incubate the cells in the dark for 1 hour with a fluorochrome-conjugated anti-rabbit secondary antibody at a concentration recommended by the provider.
9. Wash the cells 3 times with TBSTX, 10 minutes each.
10. Examine the staining under a fluorescent microscope equipped with the appropriate filter.



Lane 1: Human platelet microsomes (20 µg)
Lane 2: Murine kidney pellet (20 µg)
Lane 3: Murine spleen supernatant (40 µg)
Lane 4: Raji cell lysate (40 µg)
Lane 5: Human recombinant SPHK1 (Sf9 lysate)

WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY: NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent *via* email to your institution.

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References

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