

APE698Hu01 100µg

Active Sphingosine Kinase 1 (SPHK1)

Organism Species: *Homo sapiens* (Human)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu148~Leu398

Tags: N-terminal His-tag

Purity: >90%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .

Original Concentration: 200µg/mL

Applications: Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.9

Predicted Molecular Mass: 29.2kDa

Accurate Molecular Mass: 32kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[**SEQUENCE**]

```
LLTNCTLLLCRRLLSPMNLLSLHTASGLRLFSVLSLAWGFIADVLDLESEKYRRLGEMRFTLG  
TFLRLAALRTYRGRLAYLPVGRVGSKTPASPVVVQQGPVDAHLVPLEEPVPSHWTVVPD  
EDFVLVLALLHSHLGSEMFAAPMGRCAAGVMHLFYVRAGVSRAMLLRLFLAMEKGRH  
MEYECPYLVVYPVVAFRLEPKDGKGVFAVDGELMVSEAVQGQVHPNYFWMVSGCVEPP  
PSWKPQQMPPPEEPL
```

[**ACTIVITY**]

Sphingosine Kinase 1 (SPHK1) is a lipid kinase that catalyzes the phosphorylation of sphingosine to generate sphingosine-1-phosphate (S1P), a potent bioactive lipid mediator regulating cell proliferation, survival, and immune responses. SPHK1 is localized in the cytosol and translocates to the plasma membrane upon activation, playing critical roles in inflammation, cancer progression, and vascular biology. Its overexpression is associated with various cancers and inflammatory diseases. Besides, N-Acylsphingosine Amidohydrolase 2 (ASAH2) has been identified as an interactor of SPHK1, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human SPHK1 and recombinant rat ASAH2. Briefly, SPHK1 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to ASAH2-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-SPHK1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37°C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant human SPHK1 and recombinant rat ASAH2 was shown in Figure 1, the EC₅₀ for

this effect is 0.06ug/mL.

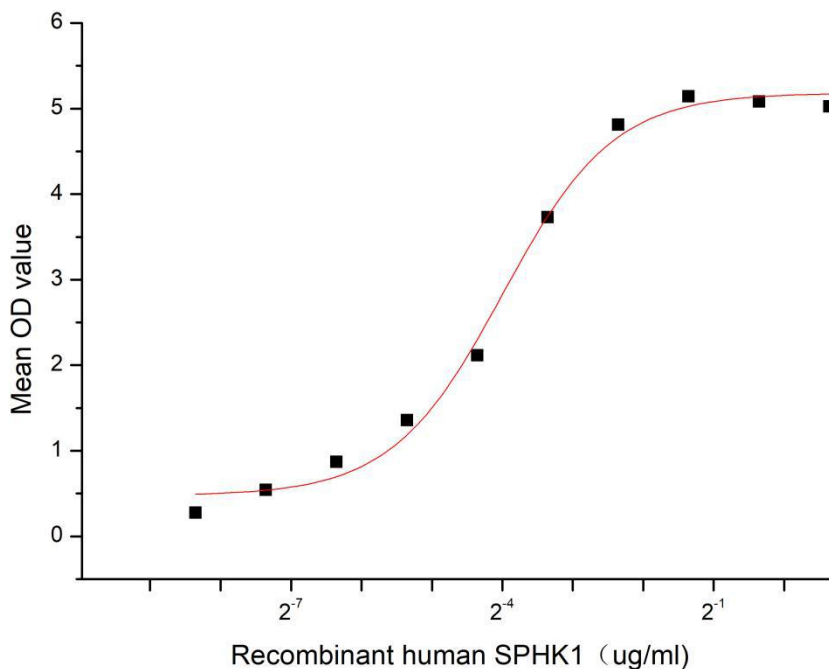


Figure 1. The binding activity of recombinant human SPHK1 and recombinant rat ASAH2

[IDENTIFICATION]

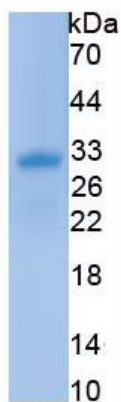


Figure 2. SDS-PAGE

Sample: Active recombinant SPHK1, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.