Active N-Acylsphingosine Amidohydrolase 2 (ASAH2)

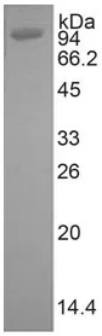
Catalog#:PHH12935

概述（Summary）

别称（Alternative Names）	HNAC1; non-lysosomal ceramidase; Neutral ceramidase
纯度（Purity）	Greater than 80% as determined by reducing SDS-PAGE.
内毒素（Endotoxin level）	Please contact with the lab for this information
蛋白构建（Construction）	【Phe99-Ile780】
Accession #	Q9NR71
表达宿主（Host）	293F
种属（Species）	Homo sapiens (Human)
预测分子量（Predicted MW）	106.0 kDa
制剂（Form）	Lyophilized from a 0.2 μm filtered solution of PBS, pH7.4, containing 0.01% SKL, 1mM DTT, 5% Trehalose and Proclin300.
运输方式（Shipping）	The product is shipped with ice bag. Upon receipt, store it immediately at the temperature listed below.
稳定性&储存（Stability &Storage）	12 months at -20°C to -80°C in lyophilized state.6 months at -20°C to -80°C under sterile conditions after reconstitution.7-10 days at 2°C to 8°C under sterile conditions after reconstitution.Please minimize freeze-thaw cycles.
复溶（Reconstitution）	Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.
背景介绍（References）	Plasma membrane ceramidase that hydrolyzes sphingolipid ceramides into sphingosine and free fatty acids at neutral pH. Ceramides, sphingosine, and its phosphorylated form sphingosine-1-phosphate are bioactive lipids that mediate cellular signaling pathways regulating several biological processes including cell proliferation, apoptosis and differentiation. Also catalyzes the reverse reaction allowing the synthesis of ceramides from fatty acids and sphingosine. Together with sphingomyelinase, participates in the production of sphingosine and sphingosine-1-phosphate from the degradation of sphingomyelin, a sphingolipid enriched in the plasma membrane of cells. Also participates in the hydrolysis of

ceramides from the extracellular milieu allowing the production of sphingosine-1-phosphate inside and outside cells. This is the case for instance with the digestion of dietary sphingolipids in the intestinal tract.

电泳图（SDS-PAGE image）



Greater than 80% as determined by reducing SDS-PAGE.

活性图（Bioactivity image）

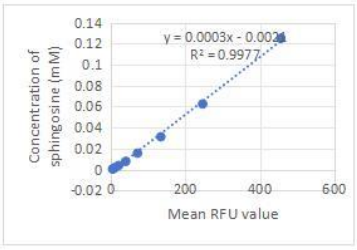


Figure 1. The standard curve of sphingosine

RFU (330/450)	Sphingosine (mM)
456.754	0.125
245.954	0.0625
133.254	0.03125
70.314	0.015625
38.694	0.0078125
19.444	0.00390625
10.124	0.001953125
5.644	0.000976563
3.163	0.000488281

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax * (RFU/min) x Conversion Factor ** (pmol/RFU)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard sphingosine

The human ASAH2 gene encodes N-acylsphingosine amidohydrolase-2, also known as neutral or non-lysosomal ceramidase. ASAH2 is a type II integral membrane protein that can be cleaved to produce a soluble secreted protein. The enzyme is abundant in the brush border membranes of the intestine, but is also expressed in tissues such as kidney, brain and liver. An N-terminally truncated form of human ASAH2 has been shown to localize to mitochondria. A major physiological function of ASAH2 is the metabolism of dietary sphingolipids, but the enzyme may also be involved in the generation of messenger molecules such as sphingosine and sphingosine 1-phosphate. The activity of recombinant human ASAH2 is measured by its ability to hydrolyze the substrate C12:0 ceramide into sphingosine and dodecanoic acid in the assay buffer 25 mM MES, 150 mM NaCl, 1% (w/v) Sodium Cholate, pH 6.5. The rhASAH2 is diluted to 0.15 ug/ml in assay buffer and 50 ul diluted rhASAH2 was loaded into a black well plate and start the reaction by adding 200 μL of 250 μM substrate, with a substrate blank containing 50 μL assay buffer and 200 μL substrate and a protein blank containing 50 ul diluted rhASAH2 and 200 ul assay buffer. Incubated at 37 ℃ for 1h, then stop reactions by heating them at 95-100 °C for 5 minutes. Add 250 μL of 2 mg/ml o-PA mixture to all reaction

vials, including controls and incubate at room temperature for 10 minutes. Load 200 µL (in duplicate) of reaction mixtures and controls in a plate and read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode. The specific activity of recombinant human ASAH2 is > 9700 pmol/min/µg.

注意事项（Note）

For Research Use Only , Not for Diagnostic Use.