

Active Acid Sphingomyelinase (ASM) Instruction Manual

SBPB225Hu61

Homo sapiens (Human)

Buffer Formulation

PBS, pH7.4, containing 0.01% SKL, 1mM DTT, 5% Trehalose and Proclin300.

Traits

Freeze-dried powder

Purity

> 95%

Isoelectric Point

7.2

Applications

Cell culture; Activity Assays.

ACTIVITY TEST

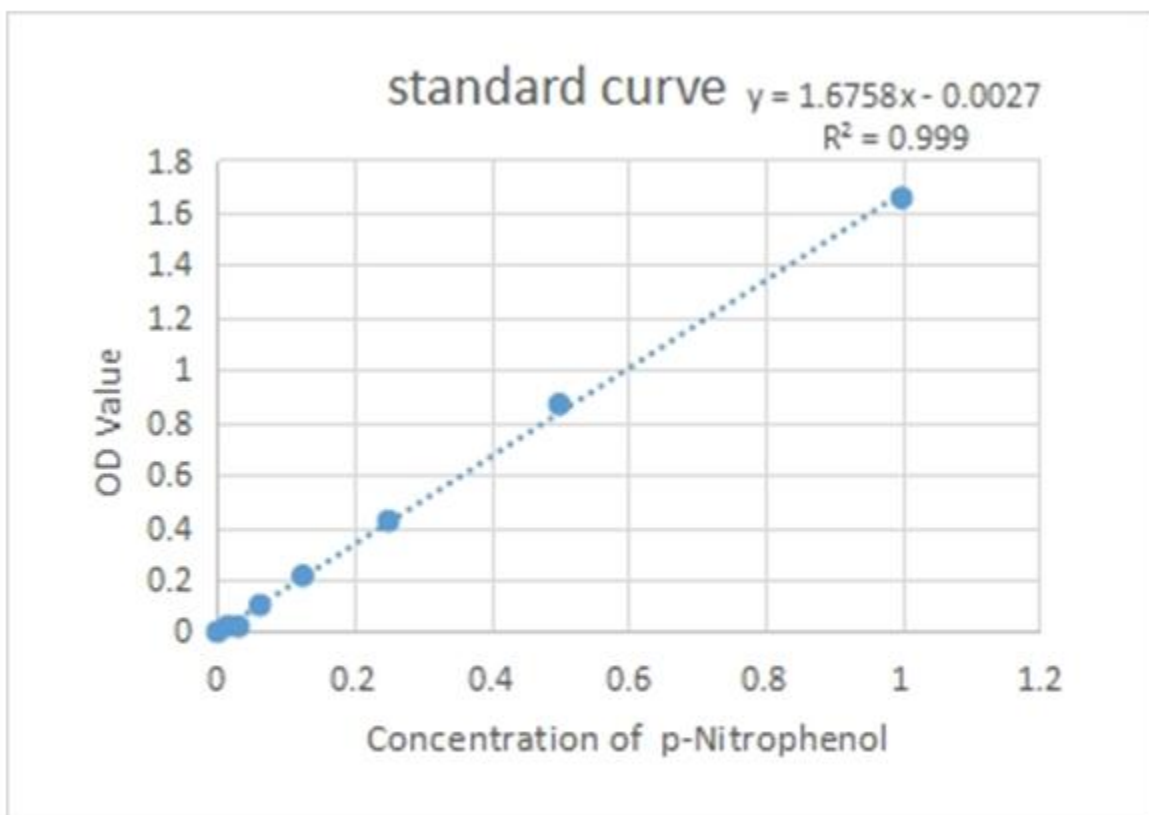


Figure 1. The standard curve of p-Nitrophenol

Sphingomyelin phosphodiesterase 1 (SMPD1), also known as acid sphingomyelinase (ASM), belongs to the sphingomyelin phosphodiesterase family. The protein Converts sphingomyelin to ceramide. ASM also has phospholipase C activities toward 1,2-diacylglycerolphosphocholine and 1,2-diacylglycerolphosphoglycerol. Thus, the recombinant human ASM activity was measured by its ability to hydrolyze 2-N-Hexadecanoylamino-4-nitrophenylphosphorylcholine (HDA-PC) to p-Nitrophenol. The reaction was performed in 50 mM MES, 0.5 μM ZnCl₂, pH 7.5 (Assay Buffer), initiated by addition 50 μL of various concentrations of ASM (diluted with Assay Buffer) to 50 μL of 1 mM Substrate HDA-PC (50 mM stock solution in methanol, diluted with Assay Buffer). Incubated at room temperature for 20 minutes in the dark, then read at a wavelength of 405 nm.

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\Delta OD * F}{T * N}$$

ΔOD=Adjusted for Substrate Blank

F=Conversion Factor(Derived using calibration standard p-Nitrophenol)

T= Time

N=Amount of enzyme

One unit of enzyme activity is defined as the 1μg of enzyme required to convert 1pmol of HDA-PC to p-Nitrophenol in 1min at 37°C. The specific activity of recombinant human ASM is >1000 pmol/min/μg.

USAGE

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

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

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.

Image

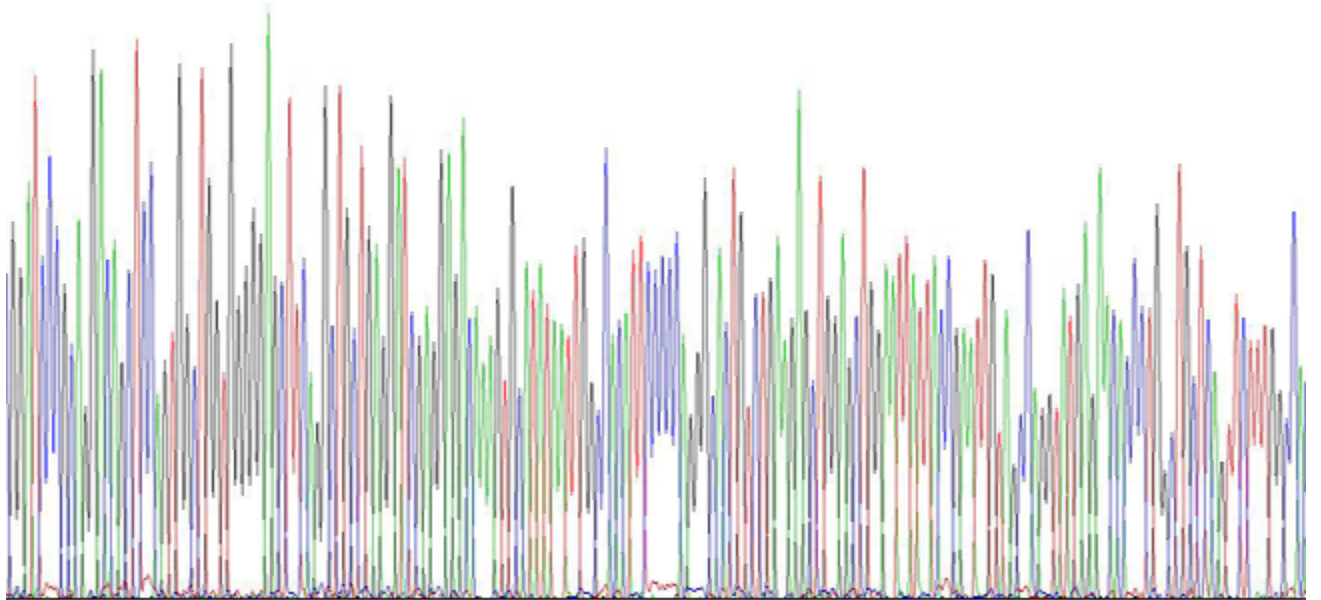


Figure. SDS-PAGE

[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.