APB360Hu61 1mg
Active Acid Sphingomyelinase (ASM)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: His62~Pro628
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.2
Predicted Molecular Mass: 64.9kDa
Accurate Molecular Mass: 75kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) 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]

HPLSPQGHP ARLHRIVPRL RDVFGWGNLT CPICKGLFTA
INLGLKKEPN VARVGSVAIK LCNLLKIAPP AVCQSIVHLF EDDMVEVWRR
SVLSPSEACG LLLGSTCGHW DIFSSWNISL PTVPKPPPKP PSPPAPGAPV
SRILFLTDLH WDHDYLEGTD PDCADPLCCR RGSGLPPASR PGAGYWGEYS
KCDLPLRTLE SLLSGLGPAG PFDMVYWTGD IPAHDVWHQT RQDQLRALTT
VTALVRKFLG PVPVYPAVGN HESTPVNSFP PPFIEGNHSS RWLYEAMAKA
WEPWLPAEAL RTLRIGGFYA LSPYPGLRLI SLNMNFCSRE NFWLLINSTD
PAGQLQWLVG ELQAAEDRGD KVHIIGHIPP GHCLKSWSWN YYRIVARYEN
TLAAQFFGHT HVDEFEVFYD EETLSRPLAV AFLAPSATTY IGLNPGYRVY
QIDGNYSGSS HVVLDHETYI LNLTQANIPG AIPHWQLLYR ARETYGLPNT
LPTAWHNLVY RMRGDMQLFQ TFWFLYHKGH PPSEPCGTPC RLATLCAQLS
ARADSPALCR HLMPDGSLPE AQSLWPRP

[ACTIVITY]

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.

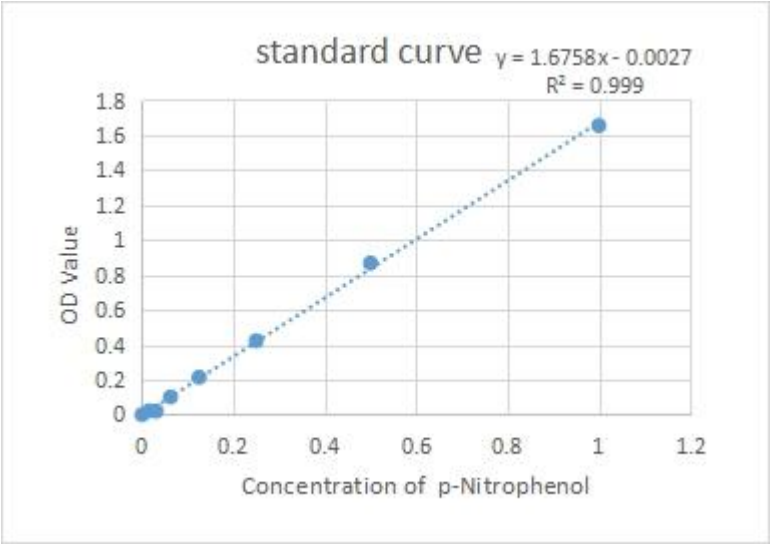

Figure 1. The standard curve of p-Nitrophenol

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.

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

△OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

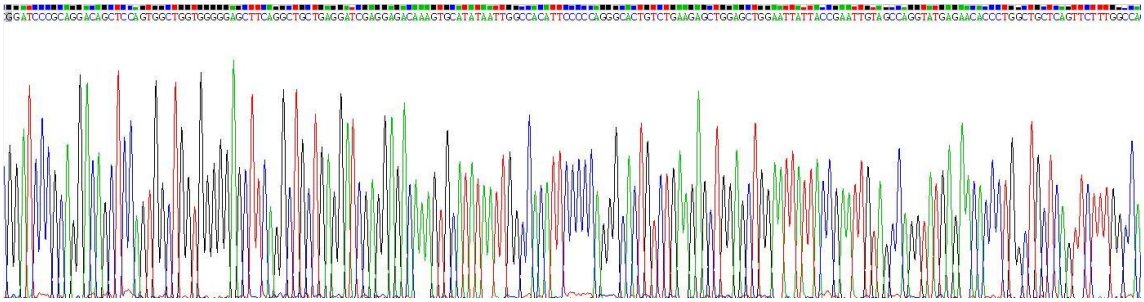

Figure 2. Gene Sequencing (extract)

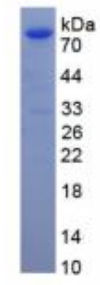

Figure 3. SDS-PAGE

Sample: Active recombinant ASM, Human

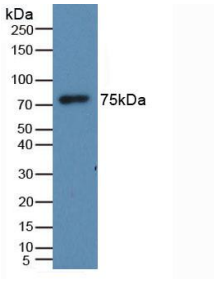

Figure 4. Western Blot

Sample: Recombinant ASM, Human;

Antibody: Rabbit Anti- Human ASM Ab (PAB360Hu06)

[IMPORTANT NOTE]

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