

**APB360Mu61 1mg**  
**Active Acid Sphingomyelinase (ASM)**  
**Organism Species: *Mus musculus (Mouse)***  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Tyr58~Leu626

**Tags:** N-terminal His-tag

**Purity:** >98%

**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:** 6.9

**Predicted Molecular Mass:** 65.1kDa

**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.

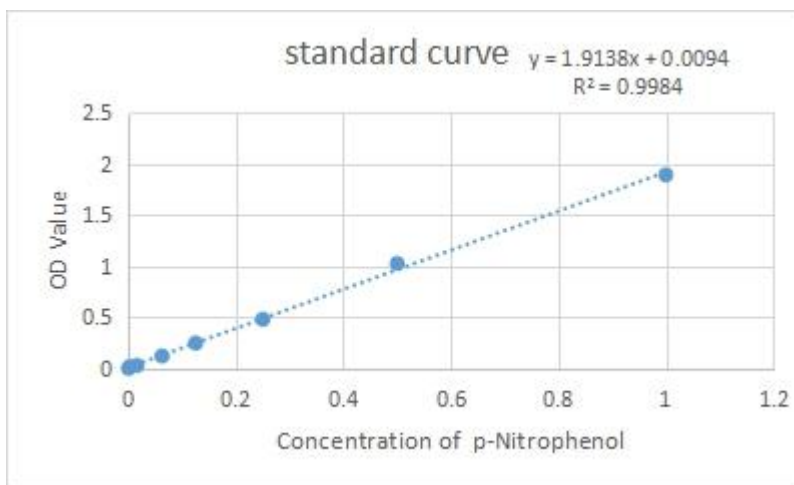
## [ SEQUENCE ]

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YPL PSEGHSVKFS AIAPPLQSAF GWQNLTCPAC KVLFTALNHG
LKKEPNVARV GSVAIKICKM LNIAPLDVCQ SAVHLFEDDV VEVWTRSVLS
PSEACGLLLG SSCGHWDIFS TWNISLPSVP KPPPKPPSPP APGAPVSRVL
FLTDLHWDHE YLEGTDPYCA DPLCCRRGSG WPPNSQKGAG FWGEYSKCDL
PLRTLLESLLK GLGPAGPFEM VYWTGDIPAH DVWQQSRQDQ LRALTTITDL
VRKFLGPPVPV YPAVGNHEST PVNGFPPPF I KGNQSSQWLY EAMAKAWEPW
LPADALHTLR IGGFYALTPR PGLRLISLNM NFCSRENFWL LINSTDPAGQ
LQWLVEELQA AENRGDKVHI IGHIPPGHCL KSWSWNYYKI IARYENTLAG
QFFGHTHVDE FEIFYDEETL SRPLAVAFLA PSATTFINLN PGYRVYQIDG
NYPGSSHVVL DHETYILNLT QANAAGGTPS WKRLYRARET YGLPDAMPAS
WHNLVYRMRD DEQLFQTFWF LYHKGHPPSE PCGTPCRLAT LCAQLSARAD
SPALCRHLMP NGSLPDANRL WSRPLL
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## [ 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-diacylglycerolphoglycerol.

Thus, the recombinant mouse ASM activity was measured by its ability to hydrolyze 2-N-Hexadecanoylamino-4-nitrophenylphosphorylcholine (HDA-PC) to p-Nitrophenol. The reaction was performed in 50mM MES, 0.5 μM ZnCl<sub>2</sub>, pH 7.5 ( Assay Buffer), initiated by addition 50μL of various concentrations of ASM (diluted with Assay Buffer) to 50 μL of 1mM Substrate HDA-PC ( 50mM 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 mouse ASM is >1000 pmol/min/μg.

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

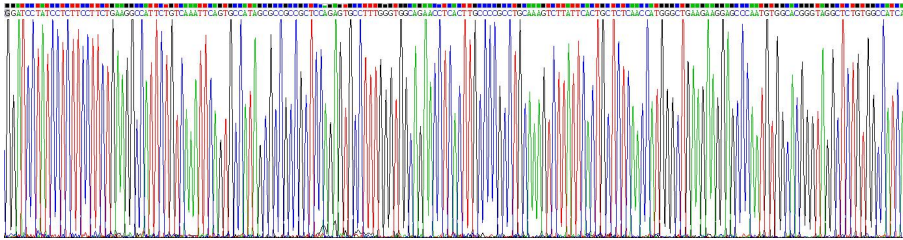
ΔOD=Adjusted for Substrate Blank

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

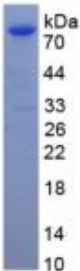
T= Time

N=Amount of enzyme

## **[ IDENTIFICATION ]**

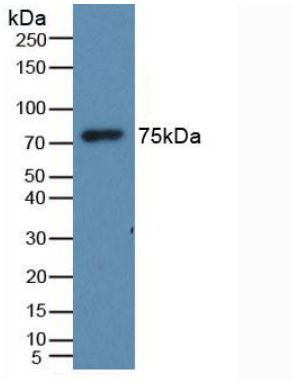


**Figure 2. Gene Sequencing (extract)**



**Figure 3. SDS-PAGE**

**Sample: Active recombinant ASM, mouse**



**Figure 4. Western Blot**

**Sample: Recombinant ASM, mouse;**

**Antibody: Rabbit Anti- mouse ASM Ab (PAB360Mu06)**

**[ 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.