APE689Hu61 100μg
Active N-Acylsphingosine Amidohydrolase 2 (ASAH2)
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: Eukaryotic expression.
Host: 293F cell
Residues: Phe99~Ile780
Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1
Purity: >80%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
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: 106.0kDa
Accurate Molecular Mass: 100kDa 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 (pH7.4) 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]

FS
GYHIGVGRAD CTGQVADINL MGYGKSGQNA QGILTRLYSR AFIMAEPDGS
NRTVFVSIDI GMVSQRLRLE VLNRLQSKYG SLYRRDNVIL SGTHTHSGPA
GYFQYTVFVI ASEGFSNQTF QHMVTGILKS IDIAHTNMKP GKIFINKGNV
DGVQINRSPY SYLQNPQSER ARYSSNTDKE MIVLKMVDLN GDDLGLISWF
AIHPVSMNNS NHLVNSDNVG YASYLLEQEK NKGYLPGQGP FVAAFASSNL
GDVSPNILGP RCINTGESCD NANSTCPIGG PSMCIAKGPG QDMFDSTQII
GRAMYQRAKE LYASASQEVT GPLASAHQWV DMTDVTVWLN STHASKTCKP
ALGYSFAAGT IDGVGGLNFT QGKTEGDPFW DTIRDQILGK PSEEIKECHK
PKPILLHTGE LSKPHPWHPD IVDVQIITLG SLAITAIPGE FTTMSGRRLR
EAVQAEFASH GMQNMTVVIS GLCNVYTHYI TTYEEYQAQR YEAASTIYGP
HTLSAYIQLF RNLAKAIATD TVANLSRGPE PPFFKQLIVP LIPSIVDRAP
KGRTFGDVLQ PAKPEYRVGE VAEVIFVGAN PKNSVQNQTH QTFLTVEKYE
ATSTSWQIVC NDASWETRFY WHKGLLGLSN ATVEWHIPDT AQPGIYRIRY
FGHNRKQDIL KPAVILSFEG TSPAFEVVTI

[ACTIVITY]

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.

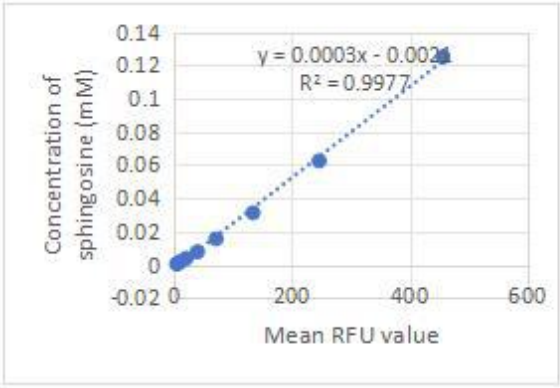

Figure 1. The standard curve of sphingosine

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

[IDENTIFICATION]

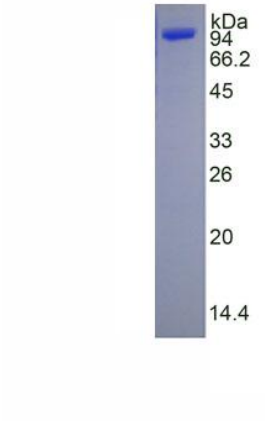

Figure 2. SDS-PAGE

Sample: Active recombinant ASAH2, 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.