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Acid Sphingomyelinase Assay Kit II (Colorimetric)

rev 04/21

(Catalog # K192-100; 100 assays; Store at -20 °C)

I. Introduction:

Sphingomyelinase (SMase) cleaves sphingomyelin to produce phosphorylcholine and ceramide. The activation of SMase leads to increased production of ceramide, which acts as a lipid second messenger. There are five distinct types of SMases. Deficiency in Acid SMase (ASMase) leads to Niemann-Pick disease type A and B. **BioVision's Acid Sphingomyelinase Assay Kit** provides a simple and sensitive method for measuring ASMase enzymatic activity using colorimetry (OD 570 nm). In this assay, ASMase converts its substrate, sphingomyelin to phosphorylcholine and ceramide at pH 5.0. Subsequently, phosphorylcholine is utilized in a series of reactions culminating in color formation from a highly specific probe. This high-throughput adaptable assay kit can detect ASMase activity as low as 0.2 mU/ml in a variety of sample types.

II. Application:

- Measurement of ASMase activity in various tissues/cell extracts

III. Sample Types:

- Animal tissues: brain, heart, kidney, etc.
- Cell culture: Adherent or suspension cells
- Serum

IV. Kit Contents:

Components	K192-100	Cap Code	Part Number
ASMase Assay Buffer I	20 ml	WM	K192-100-1
ASMase Assay Buffer II	15 ml	NM	K192-100-2
ASMase Substrate (Lyophilized)	1 vial	Blue	K192-100-3
ASMase Enzyme Mix I (Lyophilized)	1 vial	Green	K192-100-4
ASMase Enzyme Mix II (Lyophilized)	1 vial	White	K192-100-5
Choline Standard (Lyophilized)	1 vial	Yellow	K192-100-6
ASMase Probe (DMSO)	0.2 ml	Red	K192-100-7
ASMase Enhancer	100 µl	Purple	K192-100-8

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom (Polypropylene microplate)
- Multi-well spectrophotometer

VII. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Read the entire protocol before performing the assay.

- **ASMase Assay Buffer I and II:** Warm ASMase Assay Buffer I and II to Room temperature (RT) before use. Mix 5 ml of ASMase Assay Buffer I and 5 ml of ASMase Assay Buffer II in a separate tube and label as **"ASMase Buffer Mix"**. Store at -20 °C.
- **ASMase Substrate (Lyophilized):** Reconstitute with 440 µl of ASMase Buffer Mix. Store at -20 °C. Keep on ice while in use. Use within two months.
- **ASMase Enzyme Mix I (Lyophilized):** Reconstitute with 220 µl of ASMase Buffer Mix. Store at -20 °C. Keep on ice while in use. Use within two months.
- **ASMase Enzyme Mix II (Lyophilized):** Reconstitute with 220 µl of ASMase Buffer Mix. Store at -20 °C. Use within two months.
- **Choline Standard (Lyophilized):** Reconstitute with 100 µl of ddH_2O to generate 50 mM Choline Standard stock. Store at -20 °C. Use within two months.
- **ASMase Probe (DMSO):** Store at -20 °C. Avoid light exposure. Warm to RT before use. Use within two months.
- **ASMase Enhancer:** Ready to use as supplied.

VIII. Acid Sphingomyelinase Assay Protocol:

1. **Sample Preparation:** Add 100 µl of ASMase Assay Buffer I to 10 mg of sample (wet weight or 1 x 10^6 cell pellet). Homogenize on ice using a Dounce homogenizer (BV Cat. # 1998). Centrifuge at 10,000 x g for 5 min. Collect the supernatant.
2. **Acid Sphingomyelinase Assay:** Add 5-10 µl of Sample supernatant into a 96-well plate. Add 1 µl of Enhancer and 4 µl of ASMase Substrate to the well. Adjust the final volume to 25 µl with ASMase Assay Buffer I. Preincubate the Samples at 37 °C for precisely 1 hr (T) to complete the reaction at pH 5.0. We recommend covering the plates to minimize evaporation. After 1 hr, add 25 µl of ASMase Buffer II to all the Samples. Incubate the 96-well plate for 10 min at 100 °C. Quick spin the plate, if sample precipitates. Transfer supernatant into fresh wells of 96-well plate.

   Notes:

   a. We recommend adding Protease Inhibitor Cocktail (BV Cat. # K271) in 1:1000 ratio while preparing the Samples.

   b. Cell & tissue lysates can be stored at -80°C for future experiments.

   c. For Unknown Samples, we suggest performing a pilot experiment & test several doses to ensure the readings are within the Standard Curve range.

d. For Samples having high background, prepare parallel well(s) containing the same amount of Sample as in the test well. Adjust the volume to 25 µl with ASMase Assay Buffer I (do not add the ASMase Substrate), preincubate at 3 7°C for precisely 1 hr & follow the rest of the ASMase assay procedure.

e. The one hour preincubation time (T) used for ASMase activity of the Samples is based upon our experience with typical concentrations of ASMase in our Samples. This may be increased or decreased depending upon ASMase activity in your Samples.

3. **Standard Curve Preparation:** Dilute Choline Standard to 0.5 mM by adding 10 µl of 50 mM Choline Standard to 990 µl of ddH_2O and mix well. Add 0, 2, 4, 6, 8 and 10 µl of the diluted 0.5 mM Choline Standard into a series of wells in 96-well plate to generate 0, 1, 2, 3, 4 and 5 nmol/well of Choline Standard. Adjust the volume to 50 µl/well with ASMase Buffer mix.

4. **Assay Development Reaction:** Mix enough reagents for the number of assays (Samples, Standards, & Background Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
ASMase Assay Buffer I	22 µl
ASMase Assay Buffer II	22 µl
ASMase Enzyme Mix I	2 µl
ASMase Enzyme Mix II	2 µl
ASMase Probe	2 µl

Mix well. Add 50 µl of Reaction Mix to each well containing the Choline Standards, Background Control and Samples. Mix and incubate for 30 min at 37 °C.

Note: Measurement of ASMase activity is a 2-step enzymatic assay and the assay development reaction incubation time does not indicate the activity of the enzyme.

5. **Measurement:** Measure the absorbance at 570 nm.

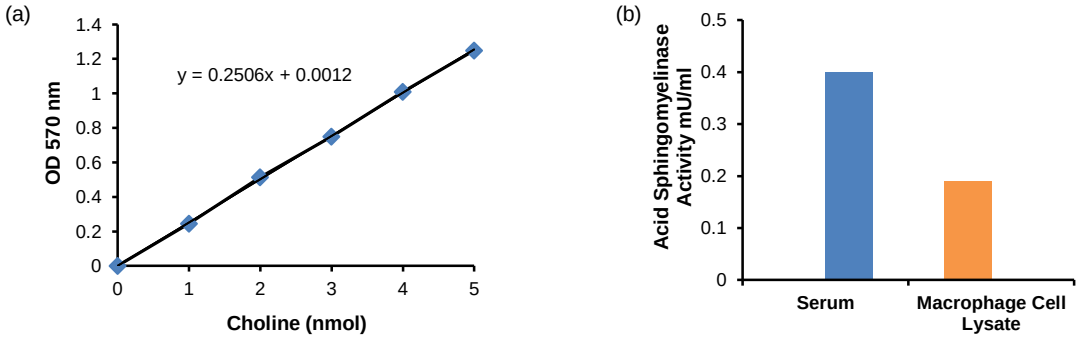
6. **Calculations:** Subtract 0 Choline Standard reading from all readings. Plot the Choline Standard Curve. If Sample Background Control reading is significant, subtract the Background Control reading from Sample readings. Calculate the ASMase activity of the Samples. Determine the OD at specific time point (T, varies depending upon the Sample type). Apply the ΔOD to the Choline Standard Curve to get B nmol of Choline generated by ASMase activity at a given time (T).

Sample Acid Sphingomyelinase Activity = B/(T X V) x D = nmol/min./ml = mU/ml

Where: **B** = Choline amount from the Standard Curve (nmol)
T = time (min.)
V = Sample volume added into the reaction well (ml)
D = Sample dilution factor

Acid Sphingomyelinase specific activity can be expressed as mU/mg of protein.

Unit Definition: One unit of ASMase activity is the amount of enzyme that generates 1.0 µmol of Choline per min. at pH 5.0 and 37°C.



Figures: (a) Choline Standard Curve, (b) ASMase activity of serum and macrophage cell lysate (1 µg/µl). Assays were performed following the kit protocol.

IX. Related Products:

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615)
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (K612)
HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613)
Triglyceride Quantification Colorimetric/Fluorometric Kit (K622)
Sphingosine Kinase Inhibitor, SKI-II (2047)
Sphingomyelin Quantification Colorimetric Assay Kit (K600-100)
Sphingomyelinase Activity Colorimetric Assay Kit (K599-100)
Protease Inhibitor Cocktail (K271)
Phosphatidylcholine Colorimetric/Fluorometric Kit (K576)
Sphingosine Kinase 1 (SPK1) Antibody (3883)
Sphingosine Kinase 2 (SPK2) Antibody (3884)
Sphingosine Kinase Inhibitor, SKI-I (2046)
C2 Ceramide (2390)
EZLys™ Mammalian Protein Extraction Reagent (8004)
Dounce Tissue Homogenizer (1998)

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