Acid Sphingomyelinase Activity Colorimetric Assay Kit

Cat. No. Kit-0040 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Acid Sphingomyelinase Activity Colorimetric Assay Kit is used to detect ASMase activity.
Description	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. Acid Sphingomyleinase Activity 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 3 mU/mL in a variety of samples.
Applications	Measurement of ASMase activity in various tissues/cell extracts. Screening of ASMase inhibitors.
Usage	For research use only (RUO)
Storage	Store the kit at -20°C, protected from light.
Kit Components	ASMase Assay Buffer I 20 mL ASMase Assay Buffer II 15 mL

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	ASMase Substrate (Lyophilized) 1 vial ASMase Enzyme Mix I (Lyophilized) 1 vial ASMase Enzyme Mix II (Lyophilized) 1 vial Choline Standard (Lyophilized) 1 vial ASMase Positive Control 10 µ ASMase Probe (DMSO) 0.2 ml
Materials Required but Not Supplied	96-well clear plate with flat bottom Multi-well spectrophotometer
Target Species	Mammals
Detection method	Colorimetric
Compatible Sample Types	Animal tissues: brain, heart, kidney,etc.Cell culture: adherent or suspension cellsSerum
Preparation	Reagent Preparation Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. ASMase Assay Buffer I and II: Warm ASMase Assay Buffer I and II to room temperature 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 4°C or -20°C. ASMase Substrate: Reconstitute with 440 µ of ASMase Buffer Mix. Store at -20°C. Keep on ice while in use. Use within two months. ASMase Enzyme Mix I: Reconstitute with 220 µ of ASMase Buffer Mix. Store at -20°C. Keep on ice while in use. Use within two months. ASMase Enzyme Mix II: Reconstitute with 220 µ of ASMase Buffer Mix. Store at -20°C. Use within two months.

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	Choline Standard: Reconstitute with 100 µ of ddH2O to generate 50 mM Choline Standard stock. Store at -20°C. Use within two months. ASMase Positive Control: Ready to use as supplied. Aliquot and store at -80°C. Use within two months. ASMase Probe (DMSO): Store at -20°C. Avoid light exposure. Warm to room temperature before use. Use within two months. Sample Preparation Add 100 µ 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. Centrifuge at 10,000 X g for 5 min. Collect the supernatant.
Assay Protocol	Acid Sphingomyelinase Assay Add 5-10 µ of sample supernatant into a 96-well plate. Add 4 µ of ASMase Substrate and adjust the volume to 25 µ with ASMase Assay Buffer I. For positive control, add 5-10 µ of ASMase Positive Control into desired well(s). Add 4 µ of ASMase Substrate and adjust the final volume to 25 µ with ASMase Assay Buffer I. Preincubate the samples & Positive Control at 37°C for precisely 1 hr (T) to complete the reaction at pH 5.0. After 1 hr, add 25 µ of ASMase Buffer II to all the samples & Positive Control. 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: 1. We recommend adding Protease Inhibitor Cocktail in 1:1000 ratio while preparing the samples. 2. Cell & tissue lysates can be stored at -80°C for future experiments. 3. For unknown samples, we suggest to do a pilot experiment & test several doses to ensure the readings are within the Standard Curve range. 4. 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 µ with ASMase Assay Buffer I (do not add the ASMase Substrate), preincubate at 37°C for precisely 1 hr & follow the rest of the ASMase assay procedure.

	5. 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. 6. The absorbance (OD 570 nm) of the Positive Control should be between 0.5-0.6. Standard Curve Preparation Dilute Choline Standard to 0.5 mM by adding 10 µ of 50 mM Choline Standard to 990 µ of ddH_2O and mix well. Add 0, 2, 4, 6, 8 and 10 µ 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 µ/well with ASMase Buffer mix. Assay Development Reaction Mix enough reagents for the number of assays ((samples, Standards, Positive Control & Background Control) to be performed. For each well, prepare 50 µ Reaction Mix containing: ASMase Assay Buffer I 22 µ ASMase Assay Buffer II 22 µ ASMase Enzyme Mix I 2 µ ASMase Enzyme Mix II 2 µ ASMase Probe 2 µ Mix well. Add 50 µ of the Reaction Mix to each well containing the Choline Standards, Positive Control, 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. Measurement Measure the absorbance (OD 570 nm).
Analysis	Subtract 0 Choline Standard reading from all readings. Plot the Standard Curve. If sample background control reading is significant, subtract background control reading

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	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 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 at 25°C.

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