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ab252889 Acid Sphingomyelinase Assay Kit (Colorimetric)

[View kit datasheet: www.abcam.com/ab252889](http://www.abcam.com/ab252889)

(use www.abcam.cn/ab252889 for China, or www.abcam.co.jp/ab252889 for Japan)

For the measurement of Acid Sphingomyelinase enzymatic activity in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Acid Sphingomyelinase Assay Kit (Colorimetric) ab252889 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 2 mU/mL in a variety of samples.

2. Protocol Summary

Prepare samples.



Add sample supernatant into a 96-well plate.



Add Enhancer and Sphingomyelin Substrate into wells, adjust with ASMase Assay Buffer and incubate at 37°C for 1 hr, at pH 5.0.



Add ASMase Buffer II to all the samples and incubate for 10 mins at 100°C. Spin plate, if precipitate forms, then transfer supernatants to fresh wells.



Prepare standards.



Prepare and add the Reaction Mix to all wells.
Incubate for 30 mins at 37°C.



Measure absorbance (OD 570 nm).



Calculate Acid Sphingomyelinase activity.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
ASMase Assay Buffer I	20 mL	-20°C	-20°C
ASMase Assay Buffer II	15 mL	-20°C	-20°C
Sphingomyelin Substrate	1 vial	-20°C	-20°C
Enzyme Mix IV	1 vial	-20°C	-20°C
Enzyme Mix II	1 vial	-20°C	-20°C
Choline Standard	1 vial	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
ASMase Enhancer	100 µL	-20°C	-20°C

PLEASE NOTE: OxiRed™ Probe was previously labelled as OxiRed Probe and ASMase Probe (DMSO). The composition has not changed.

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 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 -20°C.

6.2 Sphingomyelin Substrate

Reconstitute with 440 µL of ASMase Buffer Mix. Store at -20°C. Keep on ice while in use. Use within two months.

6.3 Enzyme Mix IV

Reconstitute with 220 µL of ASMase Buffer Mix. Store at -20°C. Keep on ice while in use. Use within two months.

6.4 Enzyme Mix II

Reconstitute with 220 µL of ASMase Buffer Mix. Store at -20°C. Use within two months.

6.5 Choline Standard

Reconstitute with 100 µL of ddH₂O to generate 50 mM Choline Standard stock. Store at -20°C. Use within two months.

6.6 OxiRed™ Probe

Store at -20°C. Avoid light exposure. Warm to room temperature before use. Use within two months.

7. Assay Procedure

- 7.1 Sample Preparation: Add 100 μL of ASMase Assay Buffer I to 10 mg of sample (wet weight or 1×10^6 cell pellet). Homogenize on ice using a Dounce homogenizer. Centrifuge at 10,000 $\times g$ for 5 mins. Collect the supernatant.
- 7.2 Acid Sphingomyelinase Assay: Add 5-10 μl of Sample supernatant into a 96-well plate. Add 1 μl of Enhancer and 4 μl of Sphingomyelin 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.
- Δ Note:** We recommend adding Protease Inhibitor Cocktail in 1:1000 ratio while preparing the samples.
- Δ Note:** Cell and tissue lysates can be stored at -80°C for future experiments.
- Δ Note:** For unknown samples, we suggest to do a pilot experiment and test several doses to ensure the readings are within the Standard Curve range.
- 7.3 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 Sphingomyelin Substrate), preincubate at 37°C for precisely 1 hr and follow the rest of the ASMase assay procedure.
- 7.4 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.

- 7.5** Standard Curve Preparation: Dilute Choline Standard to 0.5 mM by adding 10 μL of 50 mM Choline Standard to 990 μL of ddH₂O 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.
- 7.6** Assay Development Reaction: Mix enough reagents for the number of assays (samples, Standards, and Background Control) to be performed. For each well, prepare 50 μL Reaction Mix containing:

	Reaction Mix (μL)
ASM Assay Buffer I	22
ASM Assay Buffer II	22
Enzyme Mix IV	2
Enzyme Mix II	2
OxiRed™ Probe	2

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

Δ 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.

- 7.7** Measurement: Measure absorbance (OD 570 nm).
- 7.8** Calculations: Subtract 0 Choline Standard reading from all readings. Plot the Standard Curve. If sample background control reading is significant, subtract 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 OD value after the 30 min incubation at 37 C to the Choline Standard Curve to get B nmol of Choline generated by ASMase activity at a given time (T).

$$\text{Sample Acid Sphingomyelinase Activity} = \frac{B}{(T \times V)} \times D = \text{nmol/min./ml} = \text{mU/ml}$$

Where:

B = Choline amount from the Standard Curve (nmol) T = time (mins)

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.

8. Typical Data

Data provided for demonstration purposes only.

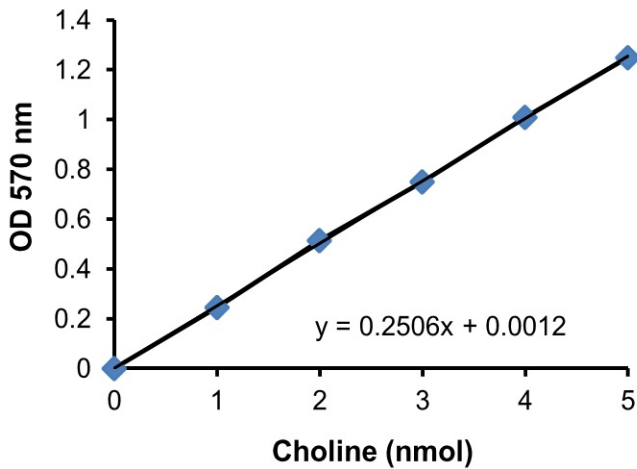


Figure 1. Choline standard curve.

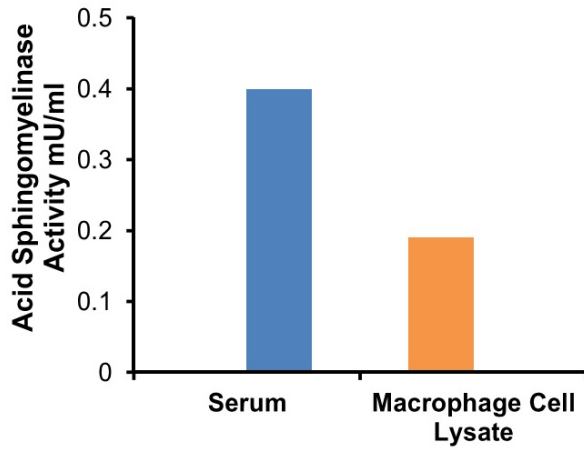


Figure 2. ASMase activity of serum and macrophage cell lysate (1 $\mu\text{g}/\mu\text{L}$).

9. Notes

Technical Support

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