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Product Information

Acid Sphingomyelinase Activity Colorimetric Assay Kit

Catalog Number **MAK188**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Acid Sphingomyelinase (ASMase) is a key enzyme in the sphingolipid pathway that hydrolyzes sphingomyelin to phosphorylcholine and ceramide. Ceramide acts as a lipid second messenger and plays a crucial role in diseases such as cystic fibrosis, diabetes, cancer, and Alzheimer's disease.[1,2]

The Acid Sphingomyelinase Activity Colorimetric Assay Kit is a simple and high throughput adaptable assay for measuring ASMase activity in a variety of tissues and cells. ASMase activity is determined by measuring a colorimetric product with absorbance at 570 nm (A_{570}) proportional to the enzymatic activity present. One unit of acid sphingomyelinase is the amount of enzyme required to generate 1.0 μmole of choline per minute at pH 5.0 at 37 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

ASMase Assay Buffer I Catalog Number MAK188A	20 mL
ASMase Assay Buffer II Catalog Number MAK188B	15 mL
ASMase Substrate Catalog Number MAK188C	1 vl
ASMase Enzyme Mix I Catalog Number MAK188D	1 vl
ASMase Enzyme Mix II Catalog Number MAK188E	1 vl
Choline Standard Catalog Number MAK188F	1 vl
ASMase Positive Control Catalog Number MAK188G	1 vl
ASMase Probe, in DMSO Catalog Number MAK188H	0.2 mL

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Protease Inhibitor Cocktail (Catalog Number P8340 or equivalent, optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

ASMase Assay Buffer I and ASMase Assay Buffer II – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use. Prepare ASMase Buffer Mix by mixing 5 mL of ASMase Assay Buffer I and 5 mL of ASMase Assay Buffer II together. Store ASMase Buffer Mix at –20 °C or 2–8 °C.

ASMase Substrate – Reconstitute with 440 μL of ASMase Buffer Mix. Dissolve completely and mix by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

ASMase Enzyme Mix I and ASMase Enzyme Mix II – Reconstitute each with 220 μL of ASMase Buffer Mix. Dissolve completely and mix by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

Choline Standard – Reconstitute with 100 µL of water to generate a 50 mM Choline Standard Solution. Aliquot and store at –20 °C. Use within 2 months.

ASMase Positive Control – Reconstitute with 100 µL of ASMase Buffer Mix. Dissolve completely and mix by pipetting. Aliquot and store at –70 °C. Use within 2 months.

ASMase Probe – Store at –20 °C. Warm to room temperature before use to melt DMSO. Aliquot and store at –20 °C.

Storage/Stability
The kit is shipped on dry ice and storage at –70 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Choline Standards for Colorimetric Detection
Dilute 10µL of 50 mM (50 nmole/µL) Choline Standard Solution with 990 µL of water and mix well to prepare a 0.5 mM (0.5 nmole/µL) Choline Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.5 mM (0.5 nmole/µL) Choline Standard Solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add ASMase Buffer Mix to each well to bring the volume to 50 µL.

Sample Preparation
Homogenize 10 mg of sample tissue with 100 µL of ice cold ASMase Assay Buffer I. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.
Note: Addition of a protease inhibitor cocktail during homogenization is recommended.

Add 5–10 µL of the sample supernatant into duplicate wells. Bring samples to a final volume of 10 µL using ASMase Assay Buffer I.
Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the ASMase Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For a positive control (optional), add 1–5 µL of the ASMase Positive Control solution to the desired wells. Adjust the final volume to 10 µL with ASMase Assay Buffer I.

Assay Reaction
1. Set up Enzymatic Reaction Mixes according to the scheme in Table 1. 15 µL of Enzymatic Reaction Mix is required for each reaction (well).

Table 1.
Enzymatic Reaction Mixes

Reagent	Controls and Samples	Sample Blank
ASMase Assay Buffer I	11 µL	15 µL
ASMase Substrate	4 µL	–

2. Add 15 µL of the appropriate Enzymatic Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 37 °C for 60 minutes.
Note: Incubation time depends on the activity of ASMase in the samples.

4. After the incubation, add 25 µL ASMase Assay Buffer II to each sample and control well. Record the total incubation time in minutes.

5. Incubate plate for 10 minutes at 100 °C. Quick spin the plate. If precipitation occurs, transfer supernatant into fresh wells.

6. Set up a Development Reaction Mix according to the scheme in Table 2. 50 µL of Development Reaction Mix is required for each reaction (well).

Table 2.
Development Reaction Mix

Reagent	Standards, Samples, Controls, and Sample Blank
ASMase Buffer Mix I	22 µL
ASMase Buffer Mix II	22 µL
ASMase Enzyme Mix I	2 µL
ASMase Enzyme Mix II	2 µL
ASMase Probe	2 µL

7. Add 50 µL of the Development Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

8. Incubate the plate at 37 °C for 30 minutes.

9. Measure the absorbance (A_{570}) in a microplate reader.
   Note: The absorbance (A_{570}) of the positive control should be 0.5–1.

Results

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) Choline Standard from that of the standards, controls, and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Choline Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank ΔA_{570} value from the Sample ΔA_{570} reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of choline (nmole/well) generated by the ASMase assay.

ASMase activity:

$$\text{ASMase Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

S_a = Amount of choline (nmole) generated in unknown sample well during the Enzymatic Reaction from standard curve

Reaction Time = length of Enzymatic Incubation (minutes)

S_v = sample volume (mL) added to well

ASMase activity is reported as nmole/min/mL = milliunit/mL.

Unit definition: One unit of acid sphingomyelinase is the amount of enzyme required to generate 1.0 μmole of choline per minute at pH 5.0 at 37 °C.

Sample Calculation:

Amount of choline (S_a) = 3.84 nmole (from standard curve)

Incubation time = 60 minutes

Sample volume (S_v) = 0.050 mL

ASMase activity in sample well:

$$\text{nmole/min/mL (milliunits/mL)} = \frac{3.84 \text{ nmole/well}}{(60 \text{ min}) \times 0.050 \text{ mL/well}} = 1.28$$

References

1. Sandhoff, K., Metabolic and cellular bases of sphingolipidoses. Biochem. Soc. Trans., **41**, 1562–1568 (2013).
2. Beckmann, N. et al., Inhibition of acid sphingomyelinase by tricyclic antidepressants and analogons. Front. Physiol., **5**, 331 (2014).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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