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ab241039 Alkaline Sphingomyelinase Assay Kit (Colorimetric)

For the detection of Alkaline Sphingomyelinase activity in tissue/cell extracts and purified enzyme preparations

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Alkaline Sphingomyelinase (AlkSMase) Assay Kit (ab241039) provides a simple, high throughput adaptable means to identify and quantify alkaline sphingomyelinase activity in a variety of samples without influence from neutral or acid sphingomyelinase. Upon incubation with substrate, the hydrolysis produces an intermediate that reacts with the OxiRed Probe/probe, generating a colorimetric signal.

The assay can detect as low as 0.5 µU of alkaline sphingomyelinase activity.

2. Protocol Summary

Prepare samples and background/positive control and add to Eppendorf tubes.

Prepare substrate mix and add to Eppendorf tubes containing positive control and test samples. Add assay buffer to background control.

Incubate all reactions at 37°C for 30-60 minutes, then boil all samples at ≥98°C for 20 mins. to stop enzymatic activity.

Place tubes on ice for 5 mins. then spin down briefly, then add samples to 96 well plate.

Prepare standard curve.

Prepare reaction mix and add to standards, positive controls and test samples.

Incubate plate for 60 min. at 37°C and read absorbance at 570 nm.

3. 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.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
AlkSMase Assay Buffer	25 mL	-20°C
AlkSMase Developer Buffer	5 mL	-20°C
OxiRed Probe/AlkSMase Probe (in DMSO)	200 µL	-20°C
Sphingomyelin Substrate/AlkSMase Substrate (Lyophilized)	1 vial	-20°C
Enzyme Mix IV/AlkSMase Enzyme Mix I (Lyophilized)	1 vial	-20°C
Enzyme Mix II/AlkSMase Enzyme Mix II (Lyophilized)	1 vial	-20°C
Choline Standard (Lyophilized)	1 vial	-20°C
AlkSMase Positive Control (Lyophilized)	1 vial	-20°C

5. Materials Required, Not Supplied

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

- 96-well plate
- Multi-well spectrophotometer

– Eppendorf tubes

6. Reagent Preparation

– Before using the kit, spin tubes and bring down all components to the bottom of tubes.
– Prepare only as much reagent as is needed on the day of the experiment.

6.1 AlkSMase Assay Buffer:

Ready to use as supplied. Bring to room temperature before use.

6.2 AlkSMase Assay Buffer:

Ready to use as supplied. Bring to room temperature before use.

6.3 OxiRed Probe/AlkSMase Probe:

Ready to use as supplied. Bring to room temperature before use. Use within 2 months.

6.4 Sphingomyelin Substrate/AlkSMase Substrate:

Reconstitute with 550 µL Assay Buffer. Gently pipette to dissolve completely. Use within two months.

6.5 Enzyme Mix IV/AlkSMase Enzyme Mix I:

Reconstitute with 220 µL Developer Buffer. Keep on ice while in use. Use within two months.

6.6 Enzyme Mix II/AlkSMase Enzyme Mix II:

Reconstitute with 1.1 mL Developer Buffer. Keep on ice while in use. Use within two months.

6.7 Choline Standard:

Reconstitute with 100 µL Assay Buffer to generate 50 mM Choline stock. Use within two months.

6.8 AlkSMase Positive Control:

Please Add 55 µL AlkSMase Assay Buffer to the Positive Control and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

7. Sample Preparation

7.1 Add 50 µL of AlkSMase Assay Buffer per 10 mg of sample (wet weight or cell pellet).
7.2 Homogenize on ice using a Dounce homogenizer.
7.3 Centrifuge at 10,000 *X g* for 5 min. at 4°C.
7.4 Collect the supernatant.
7.5 Add 5-10 µl of supernatant into an Eppendorf tube (0.6 or 1.5 ml) and adjust the volume to 50 µL with AlkSMase Assay Buffer.
7.6 For each sample, prepare identical background control reaction in a separate tube.
7.7 For positive control: add 5 µL of AlkSMase Positive Control into an Eppendorf tube and adjust the final volume to 50 µL with AlkSMase Assay Buffer.
7.8 10 µL serum samples can be directly added into each well.
7.9 Make test samples to 10 µL/well with AlkSMase Assay Buffer in a 96 well plate.

Δ Note: We recommend adding Protease Inhibitor Cocktail at a 2X final concentration while preparing the samples.

Δ Note: Cell and tissue lysate samples can be stored at -80°C for future experiments.

Δ Note: For unknown samples, we recommend doing a pilot experiment testing several doses to ensure that readings are within the range of the standard curve.

Δ Note: For samples exhibiting significant background (i.e. tissue lysates), prepare parallel sample reactions without the Sphingomyelin Substrate/substrate (sphingomyelin) as background controls.

Δ Note: We recommend filtration of small molecules that may interfere with the assay. This can be accomplished by concentrating with a10k spin column. Spin a desired volume to concentrate the protein, then dilute the ultraconcentrate back to the original volume with fresh AlkSMase Assay Buffer.

8. Assay Procedure

8.1 Add 5 µL of the Resuspended Sphingomyelin Substrate/AlkSMase Substrate to each reaction tube containing positive control or sample.

8.2 Add 5 µL AlkSMase Assay Buffer to the background control for each sample tested.

	Sample Activity Reaction Mix µL	Sample Background Reaction Mix µL
AlkSMase Reaction	50	50
AlkSMase Assay Buffer	---	5
Sphingomyelin Substrate/AlkSMase Substrate	5	---

8.3 Incubate all reactions at 37°C for 30-60 minutes.

8.4 For a positive control, run assay for 1 hr.

8.5 After desired length of time, boil all samples at ≥ 98°C for 20 minutes to stop any enzymatic activity.

8.6 Place the tubes on ice for 5 minutes.

8.7 Spin down the tubes briefly and add 50 µL of each sample to an individual well in a 96-well clear flat-bottom plate.

Δ Note: For samples with low AlkSMase Activity, longer incubation times (> 1 hr.) may be required.

9. Standard Preparation

– Always prepare a fresh set of standards for every use.

9.1 Dilute Choline Standard to 0.5 mM by adding 10 µL of 50 mM Choline Standard into 990 µL AlkSMase Assay Buffer.

9.2 Add 0, 2, 4, 6, 8 and 10 µL of the diluted Choline Standard into a series of wells in a 96-well plate to generate 0, 1, 2, 3, 4, and 5 nmol per well of Choline Standard.

9.3 Bring the total volume in each well to 50 µL with Assay Buffer.

Standard #	Choline 0.5 mM Standard (µL)	AlkSMase Assay Buffer (µL)	Choline Standard nmol/well
1	0	50	0
2	2	48	1
3	4	46	2
4	6	44	3
5	8	42	4
6	10	40	5

10. Reaction Mix Preparation

10.1 Mix enough reagents for the number of assays to be performed, including standards.

10.2 For each well, prepare 50 µL Mix containing:

	Reaction Mix µL
AlkSMase Developer Buffer	36
Enzyme Mix IV/AlkSMase Enzyme Mix I	2
Enzyme Mix II/AlkSMase Enzyme Mix II	10
OxiRed Probe/AlkSMase Probe	2

10.3 Mix and add 50 µL of the Reaction Mix to each well containing the Choline Standards, Positive Control and test samples. Mix well.

10.4 Incubate plate for 60 min. at 37°C and read absorbance at 570 nm.

11. Data Analysis

11.1 Subtract 0 Standard reading from all readings.

11.2 Plot the Choline Standard Curve.

11.3 If sample background control reading is significant, subtract the background control reading from its paired sample reading.

11.4 Calculate the alkaline sphingomyelinase activity of the test sample:

$\Delta OD = OD_{final} - OD_{initial}$.

11.5 Apply the ΔOD to the Choline Standard Curve to get B nmol of choline generated during the reaction time ($\Delta t = t2 - t1$).

Sample AlkSMase Activity = $\{B/(\Delta T \times V)\} \times D$ = nmol/min/mL = mU/mL

Where:

B is Choline amount in the sample well from Standard Curve (nmol).
ΔT is reaction time (min).

V is sample volume added into the reaction well (mL)

D is sample dilution factor (D=1 when samples are undiluted)

Unit Definition: One unit of alkaline sphingomyelinase is the amount of enzyme that generates 1.0 nmol of choline per min. at pH 9.0 at 37°C.

12. Typical Data

Typical data provided **for demonstration purposes only**.

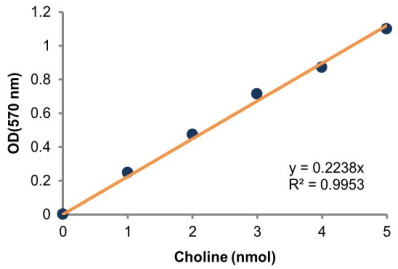

Figure 1. Choline Standard Curve.

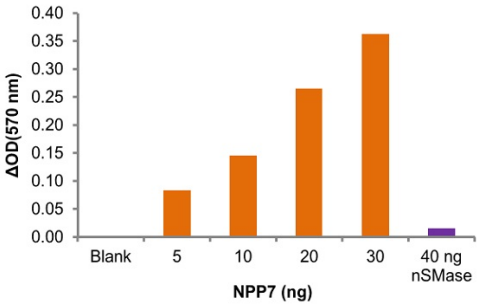

Figure 2. Alkaline Sphingomyelinase activity of NPP7. Hydrolysis was allowed to proceed for 1 hr. Neutral Sphingomyelinase (nSMase, purple bar) showed minimal activity after the same incubation time.

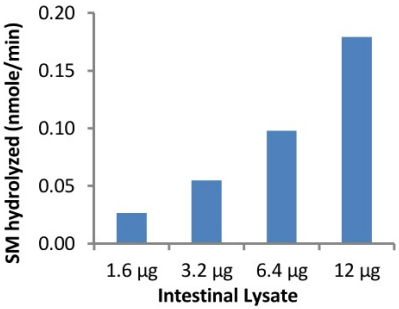

Figure 3. Activity determination of intestinal tissue lysate. For this experiment, 100 mg rat intestine was used. Lysate was assayed and specific activity was determined to be 0.016 nmol/min/µg lysate.

13. Notes

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