

Product Information

Sarcosine Assay Kit

Catalog Number **MAK073**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

Sarcosine, also known as N-methylglycine, is an amino acid that participates in one-carbon metabolism, serving as a one-carbon donor in mitochondrial reactions. Elevated levels of sarcosine may indicate deficiencies in Vitamin B₁₂ and/or folate. Sarcosine is also an intermediate in glycine and choline metabolism. In the brain, sarcosine acts as an inhibitor of the type I glycine transporter, resulting in increased glycine levels and NMDA receptor activation suggesting it may be a therapeutic agent for the treatment of some mental illnesses such as schizophrenia, depression, or obsessive compulsive disorder.

In this assay, sarcosine concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric ($\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587 \text{ nm}$) product, proportional to the sarcosine present. This kit is suitable for sarcosine detection in cell and tissue culture supernatants, plasma, serum, and other biological samples. Urine samples are not suitable for use with this assay because of sample interferences.

Components

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

Sarcosine Assay Buffer Catalog Number MAK073A	25 mL
Sarcosine Probe, in DMSO Catalog Number MAK073B	0.2 mL
Sarcosine Enzyme Mix Catalog Number MAK073D	1 vL
Sarcosine Standard, 10 μmole Catalog Number MAK073E	1 vL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

Sarcosine Assay Buffer – Allow buffer to come to room temperature before use.

Sarcosine Probe – Warm to room temperature to thaw solution prior to use. Aliquot and store protected from light and moisture at -20°C . Upon thawing, the Sarcosine Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Sarcosine Probe Solution 5 to 10-fold with Sarcosine Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Sarcosine Enzyme Mix – Reconstitute in 220 μL of Sarcosine Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light at -20°C . Use within 2 months of reconstitution. Solution is sensitive to freeze/thaw cycles and should be stored in single-use aliquots.

Sarcosine Standard – Reconstitute in 100 μL of water to generate a 100 mM (100 nmole/ μL) stock solution. Mix well by pipetting, then aliquot and store at $-20\text{ }^{\circ}\text{C}$. Keep cold while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at $-20\text{ }^{\circ}\text{C}$, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Sarcosine Standards for Colorimetric Detection

Dilute 10 μL of the 100 mM (100 nmole/ μL) Sarcosine Standard Solution with 990 μL of Sarcosine Assay Buffer to prepare a 1 mM (1 nmole/ μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM Sarcosine standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Sarcosine Assay Buffer to each well to bring the volume to 50 μL .

Sarcosine Standards for Fluorometric Detection

Dilute 10 μL of the 100 mM (100 nmole/ μL) Sarcosine Standard Solution with 990 μL of Sarcosine Assay Buffer to prepare a 1 mM (1 nmole/ μL) standard solution. Dilute 10 μL of the 1 mM standard solution with 90 μL of Sarcosine Assay Buffer to generate a 0.1 mM (0.1 nmole/ μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.1 mM Sarcosine standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Sarcosine Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Liquid samples like serum and plasma can be measured directly. Urine samples are not suitable for use with this assay because of sample interferences.

Tissue (10 mg) or cells (2×10^6) can be homogenized in 100 μL of the Sarcosine Assay Buffer. Centrifuge the samples at $13,000 \times g$ for 10 minutes to remove insoluble material.

Bring samples to a final volume of 50 μL with Sarcosine Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.

Reaction Mix

Reagent	Volume
Sarcosine Assay Buffer	46 μL
Sarcosine Enzyme Mix	2 μL
Sarcosine Probe	2 μL

2. Add 50 μL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at $37\text{ }^{\circ}\text{C}$. Cover the plate tightly and protect the plate from light during the incubation.
3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity ($\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587\text{ nm}$).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Sarcosine Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Sarcosine standards to plot a standard curve.

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

Concentration of Sarcosine

$$S_a/S_v = C$$

S_a = Amount of Sarcosine in unknown sample (nmole)
from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Sarcosine in sample

Sarcosine molecular weight: 89.1 g/mole

Sample Calculation

Amount of Sarcosine (S_a) = 5.84 nmole
(from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Sarcosine in sample

$$5.84 \text{ nmole}/50 \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 89.1 \text{ ng/nmole} = 10.41 \text{ ng}/\mu\text{L}$$

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 fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Sarcosine Assay Buffer
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used 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 Master Reaction Mix 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 mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix 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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