USER GUIDE

For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

肌氨酸检测试剂盒

Sarcosine Assay Kit

Do not eat Store at -20° C & in the dark.

Cat.No.MSC3097 **Size :** 100 tests

Technical literature is available at : www.mesgenbio.com
E-mail MesGen Technical Services if you have questions on use of this system : tech@mesgenbio.com

Application

Sarcosine assay kit provides a reliable, sensitive assay for the quantification of sarcosine in biological samples.

Description

Sarcosine, a natural amino acid, plays important roles as intermediate in the metabolism of choline, methionine, glycine, glutathione, creatine, purine and serine, etc. Detection of sarcosine level has wide applications in research and development. MesGen Biotechnology's Sarcosine Assay Kit provides an accurate, convenient measure of sarcosine in variety biological samples. In the assay, sarcosine is specifically oxidized to generate a product that converts a colorless probe to a product with intense red color (λmax = 570 nm) and which is also highly fluorescent (Ex/Em = 538/587 nm). Sarcosine is therefore easily detected by either colorimetric or fluorometric methods with detection range 0.5-2500 μM.

Key Features

Sensitive and accurate. Use as little as 20 μL samples. Linear detection range in 96-well plate: 1 to 2500 μM sarcosine for colorimetric assays and 0.5 to 250 μM for fluorimetric assays.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5-10 min at room temperature.

Samples

Serum, plasma, urine, saliva, cell culture medium, food, tissue extracts, agriculture etc

Kit contents

Reagent A : 5 mL
Reagent B : 5 mL
Reagent C : 200 μL
20mM Sarcosine Standard: 1 mL

Coloriemtric procedure

Note:
(1) *glycerol and SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.*
(2) *This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.*

1. Equilibrate all components to room temperature. During experiment, keep reconstituted Reagent in a refrigerator or on ice.
2. *Standards and samples*: prepare 500 μL 2000 μM Standard by mixing 50 μL 20 mM standard with 450 μL dH_2O. Dilute standard in dH_2O as follows.

No.	2000 μM STD + H_2O	Vol (μL)	Sarcosine (μM)
1	100 + 0	100	2000
2	80 + 20	100	1600
3	60 + 40	100	1200
4	40 +60	100	800
5	10 + 90	100	200
6	5 + 95	100	100
7	2 + 98	100	40
8	0.5 + 95.5	100	10
9	0 + 100	100	0

Transfer 20 μL standards and 20 μL samples into separate wells of a clear flat-bottom 96-well plate.

3. *Reaction*. For each reaction well, mix 50 μL Reagent A, 50 μL Reagent B and and 2 μL Reagent C (*vortex briefly before pipetting*). Transfer 100 μL Working Reagent into each reaction well. Tap plate to mix. Incubate 5-10 min at room temperature.
4. Read optical density at 570nm (550-585nm).

Fluorimetric procedure

For fluorimetric assays, the linear detection range is 0.5 to 250 μM sarcosine. Prepare 200 μM sarcosine standard by mixing 5 μL 20 mM standard with 495 μL H_2O.
Then dilute standards in H_2O (see *Colorimetric Procedure*) to 200, 150, 100, 50, 10, 1, 0.5 and 0 μM.

1. Transfer 20 μL standards and 20 μL samples into separate wells of a *black* 96-well plate.

2. Add 100 µL Working Reagent, tap plate to mix. Incubate 5-10 min.
3. Read fluorescence at λex = 530nm and λem = 585nm.

Notes: If the calculated sarcosine concentration of a sample is higher than 2500 µM in colorimetric assay or 250 µM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

Calculation

Subtract blank value from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the sarcosine concentration of Sample,

$$\textit{Colorimetry: } [\text{sarcosine}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

$$\textit{Fluorimetry: } [\text{sarcosine}] = \frac{RFU_{SAMPLE} - RFU_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

OD_{SAMPLE}, OD_{H2O} are optical density values of the sample and water.RFU_{SAMPLE}, RFU_{H2O} are fluorescence intensity values of the sample and water. n is the dilution factor.

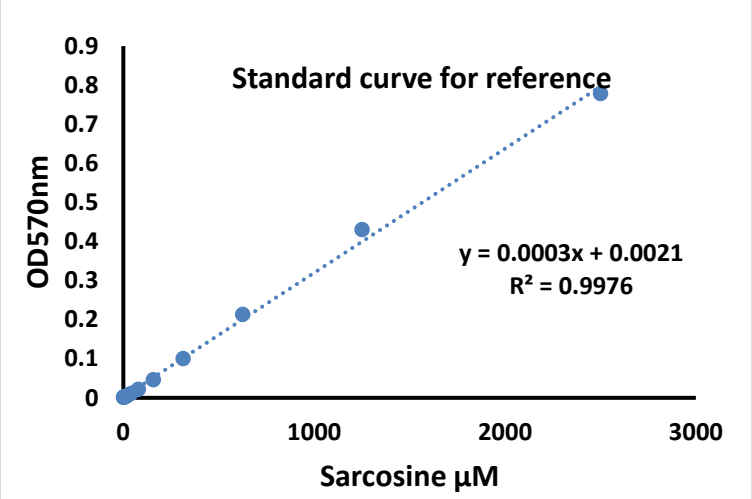
Conversions: 20 mM sarcosine equals 1.7818 mg/mL.

Storage conditions

The kit is shipped on dry ice. Store all components at -20°C.

Shelf life

6 months after receipt.



Precautions

Reagents are for research use only.

Normal precautions for laboratory reagents should be exercised while using the reagents.

Please refer to Material Safety Data Sheet for detailed information.

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