Product Manual

Sarcosine Assay Kit

Catalog Number

MET-5072 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Sarcosine is an intermediate in glycine synthesis: sarcosine is converted to glycine by the enzyme sarcosine dehydrogenase. Glycine-N-methyl transferase can convert glycine to sarcosine as a byproduct. Additionally, sarcosine is an intermediate in the production glycine from dietary consumption of choline. Sarcosine, an amino acid derivative also known as N-methyl glycine, is found in various tissues throughout the body. It is used in making toothpastes and biodegradable surfactants. Sarcosine can be found in foods such as vegetables, ham, egg yolks, turkey and legumes. The normal concentrations of sarcosine in human serum and in human urine are 1.4 µM and 1.6 µM respectively.

Sarcosine has been observed to improve treatment of the mental illness known as schizophrenia: 2 g per day consumption of sarcosine (in addition to antipsychotic drug therapy) results in significant additional reductions not only both positive and negative symptoms, but also general psychopathological and neurocognitive symptoms. Sarcosine is thought to work by increasing glycine concentrations in the brain which causes increased NMDA receptor activation. Interestingly, consumption of sarcosine also reduced depressive symptoms in patients with schizophrenia. A clinical study showed sarcosine to be significantly more effective in treating major depression than the established antidepressant drug Citalopram.

Cell Biolabs' Sarcosine Assay Kit is a simple fluorometric assay that measures the amount of sarcosine present in foods or biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, sarcosine standards and unknown samples. Sample sarcosine concentrations are determined by comparison with a known sarcosine standard. The kit has a detection sensitivity limit of 3 µM sarcosine.

Assay Principle

Cell Biolabs' Sarcosine Assay Kit measures sarcosine within food or biological samples. Sarcosine is converted by sarcosine oxidase into glycine plus formaldehyde and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are read with a standard 96-well fluorometric plate reader. Samples are compared to a known concentration of sarcosine standard within the 96-well microtiter plate format (Figure 1).

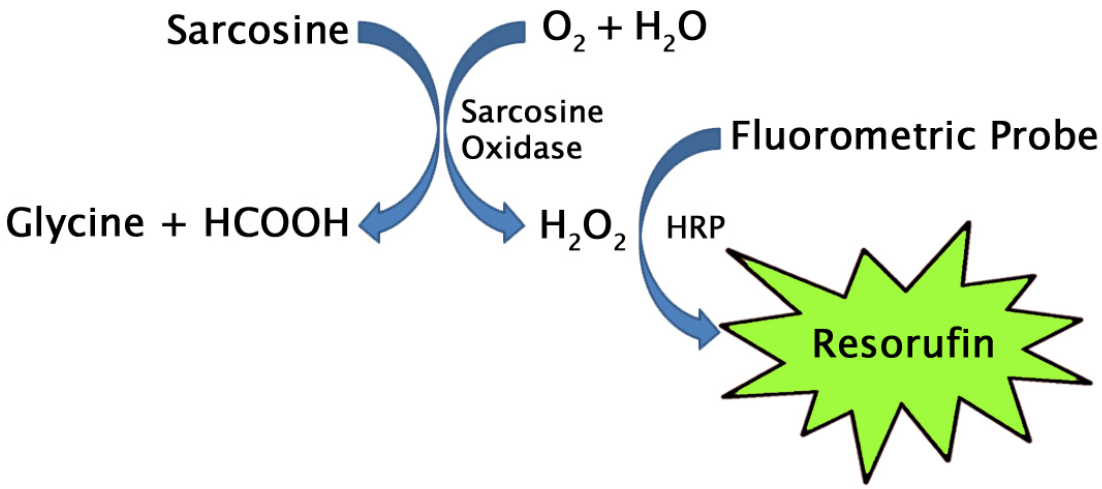

Figure 1. Sarcosine Assay Principle.

Related Products

1. MET-5070: Glycine Assay Kit
2. MET-5071: Taurine Assay Kit
3. STA-631: Total Bile Acid Assay Kit (Colorimetric)
4. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
5. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
6. STA-670: Homocysteine ELISA Kit
7. STA-671: S-Adenosylhomocysteine (SAH) ELISA Kit
8. STA-672: S-Adenosylmethionine (SAM) ELISA Kit
9. STA-674: Glutamate Assay Kit
10. STA-675: Hydroxyproline Assay Kit

Kit Components

1. Sarcosine Standard (Part No. 50721C): One 50 µL tube at 20 mM.
2. 10X Assay Buffer (Part No. 234403): One 25 mL bottle.
3. Fluorometric Probe (Part No. 50231C): One 50 µL amber tube.
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol.
5. Sarcosine Oxidase (Part No. 50722D): One 250 µL tube containing 40 U/mL Sarcosine Oxidase from *Bacillus sp*.

   Note: One unit is defined as the amount of enzyme that will convert 1.0 µmole of sarcosine to glycine and formaldehyde per minute at pH 8.3 and 37°C.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Microcentrifuge tubes
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Standard 96-well black microtiter plate and/or cell culture microplate
7. Multichannel micropipette reservoir
8. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at 4ºC. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Stir to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and Sarcosine Oxidase 1:20 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 50 µL of Sarcosine Oxidase to 938 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Urine, Serum or Plasma: Not recommended since normal sarcosine levels are below the sensitivity level for this kit.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this*

interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).

- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Sarcosine Standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	20 mM Sarcosine Solution (µL)	1X Assay Buffer (µL)	Sarcosine (µM)
1	5	495	200
2	250 of Tube #1	250	100
3	250 of Tube #2	250	50
4	250 of Tube #3	250	25
5	250 of Tube #4	250	12.5
6	250 of Tube #5	250	6.25
7	250 of Tube #6	250	3.13
8	0	250	0

Table 2. Preparation of Sarcosine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each Sarcosine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of sarcosine within samples by comparing the sample fluorescence to the standard curve.

Example of Results

The following figures demonstrate typical Sarcosine Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

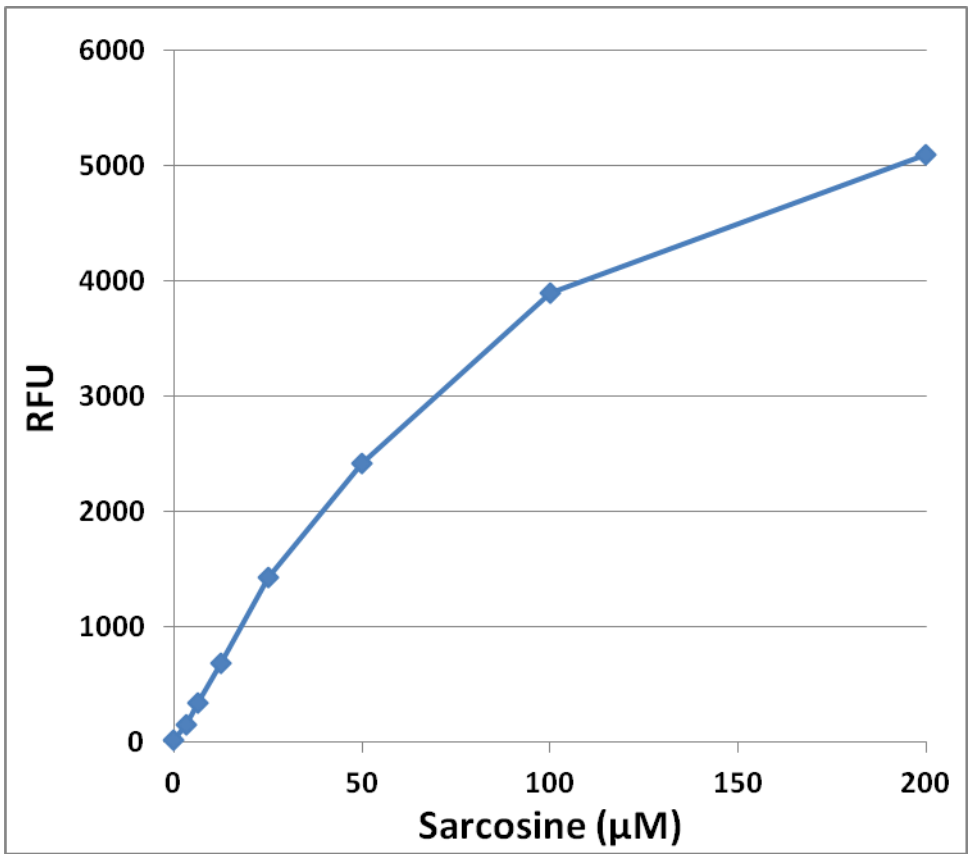

Figure 2: Sarcosine Standard Curve.

References

1. Allen RH, Stabler SP, Lindenbaum J 1993). *Metabolism*. **42**: 1448–60
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.
3. Chakraborty E and Deshpande K. (2014) *J. Pharm Biol. Sci.* **9**:49-52.
4. Lane HY, Huang CL, Wu PL, Liu YC, Chang YC, Lin PY, Chen PW, Tsai G (2006). *Biological Psychiatry*. **60**: 645–9
5. Huang CC, Wei IH, Huang CL, Chen KT, Tsai MH, Tsai P, Tun R, Huang KH, Chang YC, Lane HY, and Tsai GE (2013). *Bio.l Psychiatry*. **74**: 734–41.
6. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C, and Chinnaiyan AM (2009). *Nature*. **457**: 910–4.
7. Jentzmik F, Stephan C, Miller K, Schrader M, Erbersdobler A, Kristiansen G, Lein M, and Jung K (2010). *European Urology*. **58**: 12–8.
8. Struys EA, Heijboer AC, van Moorselaar J, Jakobs C, and Blankenstein MA (2010). *Annals Clin. Biochem*. **47**: 282.
9. Pavlou M, Diamandis EP (2009). *Clin. Chem.* **55**: 1277–9.

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