Sarcosine Detection Kit

Cat. No. KITG-148 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Sarcosine Detection Kit is designed for rapid and highly sensitive quantification of sarcosine by fluorescence or absorbance detection in biological fluids such as serum and plasma, tissues, cells, and tissue or cell culture supernatants. Usually, 0.2–2 µL of serum or plasma is sufficient for fluorescence detection.
Species	Mouse, rat, human, etc.
Description	Amplex Red is a highly sensitive fluorescent probe for H_2O_2. In the presence of horseradish peroxidase (HRP), Amplex Red reacts with H_2O_2 at a 1:1 ratio to generate the strongly red-fluorescent product resorufin. Resorufin has a maximum excitation wavelength of 571 nm and a maximum emission wavelength of 585 nm, and exhibits strong visible-light absorption at the excitation wavelength. Sarcosine is oxidized by sarcosine oxidase (SOX or SAO) in the presence of oxygen to produce formaldehyde, glycine, and H_2O_2. The resulting H_2O_2 reacts with Amplex Red, and the fluorescence intensity or absorbance of resorufin is proportional to the sarcosine content of the sample.
Form	Size 100 assays Kit Components Lysis Buffer A for Metabolic Assay — 20 mL Sarcosine Assay Buffer — 20 mL Amplex Red — 200 µL Enzyme Solution A — 200 µL Enzyme Solution B — 200 µL Sarcosine Standard (5 mM) — 200 µL Instruction manual — 1 copy
Bio-activity	Sensitivity With a 20 µL sample volume, absorbance detection can detect sarcosine at concentrations as low as 5 µM, and fluorescence detection can detect sarcosine at

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	concentrations as low as 0.5 μM.
Applications	Applications Quantitative detection of sarcosine in serum, plasma, cell culture supernatants, tissue samples, and cell samples from mouse, rat, human, and other species; widely used in research and drug development. Detection Method Absorbance detection at A570 or fluorescence detection with excitation at 560 nm and emission at 590 nm Compatible Sample Types Serum, plasma, cell culture supernatant, tissue samples, and cell samples
Notes	Notes Amplex Red is not very stable in air; after opening, use it as soon as possible a nd protect it from light during use. The reaction product of Amplex Red is unstable in t he presence of reducing agents; therefore, the concentration of dithiothreitol (DTT), β-mercaptoethanol, or similar reducing agents in the final reaction system should be bel ow 10 μM. Ensure that the pH of the reaction system is between 7 and 8, otherwise th e stability of Amplex Red and the fluorescence value will be affected. Urine interferes with the assay, so urine samples are not suitable for this kit. Amplex Red and Sarcosi ne Assay Buffer must be completely thawed and equilibrated to room temperature bef ore use; otherwise, test results may be affected. All other solutions should be kept on ice during use. Under tested conditions, the sarcosine standard curve and linear rang e are generally consistent with the description in this manual; however, actual perform ance may vary with experimental conditions and instruments. If the highest-concentra tion point is abnormally low or outside the linear range, it can generally be excluded, a nd the standard curve can be fitted using data within the linear range. To minimize err ors caused by fluorescence background from the diluent, select the diluent according to the sample type: use Lysis Buffer A for Metabolic Assay for cell or tissue lysates pr epared with this buffer, and use Sarcosine Assay Buffer for blood and other samples t hat do not require processing. Serum and plasma stored at 4°C should not be kept for more than 2 weeks. Serum samples are usually stored at -20°C, and storage at -80°C is preferable. For fluorometric detection, use a black or white plate suitable for fluores cence detection. Keywords Sarcosine; N-methylglycine; Amplex Red; resorufin; H

$_2O_2$; sarcosine oxidase; colorimetric assay; fluorometric assay Scientific Background Sarcosine, also known as N-methylglycine or 2-(methylamino)acetic acid, is a naturally occurring amino acid derivative found in muscle and other tissues. Its molecular formula is $C_3H_7NO_2$ and its molecular weight is 89.09. Sarcosine is a natural, non-genetically encoded amino acid involved in one-carbon metabolism and can serve as a one-carbon donor in mitochondrial reactions. It is an intermediate in the conversion of choline to glycine and in metabolic pathways involving methionine, glycine, glutamine, creatine, purines, and serine, and therefore plays an important role in metabolism. Elevated sarcosine levels may indicate vitamin B12 and/or folate deficiency. In the brain, sarcosine can act as an inhibitor of the type-1 glycine transporter, thereby increasing glycine levels and activating NMDA receptors, suggesting that sarcosine may have potential as a therapeutic agent for certain psychiatric disorders such as schizophrenia, depression, or obsessive-compulsive disorder. Technical Notes Enzyme solutions are used in small volumes and may settle; gently centrifuge and mix thoroughly before use. H_2O_2 interferes with sarcosine detection. If a sample contains H_2O_2, prepare a sample background control with Amplex Red Working Solution lacking Enzyme Solution A by replacing Enzyme Solution A with Sarcosine Assay Buffer, and subtract the background control reading from the sample reading. If absorbance or fluorescence is weak, the reaction time may be extended to 45 or 60 minutes. To ensure sample values fall within the standard curve range, perform a preliminary experiment with several sample dilutions to estimate the sarcosine concentration. If values are outside the standard curve range, adjust the sample dilution or increase the sample volume. Features & Benefits High detection sensitivity, a broad linear range, and low sample consumption. With a sample volume of 20 µL, absorbance detection can detect sarcosine at concentrations as low as 5 µM with good linearity from 5 to 500 µM; fluorescence detection can detect sarcosine at concentrations as low as 0.5 µM with good linearity from 0.5 to 100 µM. Fluorescence detection is approximately 1

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	0-fold more sensitive than absorbance detection and requires less sample. Flexible detection methods and rapid assay speed: both fluorescence and absorbance detection can be used, and the entire detection process can be completed in about 1 hour. The included lysis buffer has broad compatibility: cell or tissue lysates prepared with Lysis Buffer A for Metabolic Assay can also be used with other metabolic assay kits that use the same lysis buffer, and the lysates can also be used for protein concentration determination, SDS-PAGE, and Western blotting of relatively easily soluble proteins. Broad application range: suitable for serum, plasma, and other biological fluids, cell culture supernatants, tissue samples, and cell samples from mouse, rat, human, and other species. Suitable for both small-sample detection and high-throughput screening using automated systems; one small package provides 100 assays in a 96-well format. Introduction This kit uses an enzyme-coupled Amplex Red/HRP detection system to measure H_2O_2 generated from sarcosine oxidation, enabling sarcosine quantification by absorbance or fluorescence detection. Handling Protect Amplex Red and Enzyme Solution B from light. Keep all other solutions on ice during use as directed. Warning For professional scientific research use only. Not for clinical diagnosis or treatment, food, or drug use. Do not store in an ordinary residence. Wear a laboratory coat and disposable gloves for safe and healthy operation.
Storage	Storage -20°C for one year. Protect Amplex Red and Enzyme Solution B from light. Stability One year when stored at -20°C.
Reaction conditions	Preparation Sample preparation: For serum samples, place whole blood at room temperature, such as 25°C, for 30 minutes to 2 hours. Do not shake vigorously to avoid hemolysis. After the blood naturally clots and serum separates, centrifuge at approximately 1,000–2,000 × g at 4°C for 10 minutes, and collect the yellow supernatant as serum without aspirating the white or light-yellow precipitate. For plasma samples, anticoagulate whole blood with heparin or EDTA, centrifuge at approximately 1,000–2,000 × g at 4°C for 10 minutes, and collect the yellow or light-

yellow supernatant as plasma without aspirating the white precipitate. Keep serum and plasma on ice. If not tested immediately, aliquot and store at -20°C or -80°C. Thaw frozen samples and keep them on ice before use, and mix thoroughly before use. For adherent cultured cells, wash once with PBS and completely remove residual liquid. For suspended cultured cells, first collect the cells by centrifugation, such as 100–500 × g for 5 minutes, discard the supernatant, and completely remove residual liquid. Add Lysis Buffer A for Metabolic Assay at 100–200 µL per 1 million cells, pipette appropriately, and lyse on ice for 5–10 minutes. Centrifuge at approximately 12,000 × g at 4°C for 3–5 minutes and collect the supernatant for subsequent detection. For tissue samples, add Lysis Buffer A for Metabolic Assay at 100 µL per 10 mg of tissue and homogenize at approximately 4°C or on ice using a high-throughput tissue homogenizer, a handheld tissue homogenizer, or a glass homogenizer. Centrifuge at approximately 12,000 × g at 4°C for 3–5 minutes and collect the supernatant for subsequent detection. Perform all operations at 4°C or on ice. Cell or tissue samples that are not tested immediately can be stored at -20°C or -80°C. For adherent cells, collect the culture medium directly as the cell culture supernatant sample; for suspended cells, centrifuge and collect the culture medium. Reagent preparation: Thaw Amplex Red and Sarcosine Assay Buffer, equilibrate them to room temperature, and mix before use. Keep all other reagents on ice before use and return them promptly to the required storage conditions after use. Prepare Amplex Red Working Solution at 80 µL per reaction. For one reaction, mix 74 µL Sarcosine Assay Buffer, 2 µL Amplex Red, 2 µL Enzyme Solution A, and 2 µL Enzyme Solution B to prepare 80 µL Working Solution. For 10 reactions, mix 740 µL Sarcosine Assay Buffer, 20 µL Amplex Red, 20 µL Enzyme Solution A, and 20 µL Enzyme Solution B to prepare 800 µL Working Solution. For 20 reactions, mix 1,480 µL Sarcosine Assay Buffer, 40 µL Amplex Red, 40 µL Enzyme Solution A, and 40 µL Enzyme Solution B to prepare 1,600 µL Working Solution. For 50 reactions, mix 3,700 µL Sarcosine Assay Buffer, 100 µL Amplex Red, 100 µL Enzyme Solution A, and 100 µL Enzyme Solution B to prepare 4,000 µL Working Solution. Prepare an

appropriate amount according to the total number of samples and standards. Working Solution stored at 4°C or on ice protected from light can be used on the same day, but fresh preparation is recommended. Assay Protocol 1. Set up the sarcosine standard curve using either absorbance or fluorescence detection; fluorescence detection is preferentially recommended when sample volume is limited or the concentration is low. For absorbance detection, mix 10 µL Sarcosine Standard (5 mM) with 90 µL of the appropriate diluent to prepare a 500 µM sarcosine standard solution. The appropriate diluent is Lysis Buffer A for Metabolic Assay for cell or tissue lysates prepared with that buffer, or Sarcosine Assay Buffer for blood, supernatant, and other samples that do not require processing. Add 0, 0.2, 0.4, 0.8, 2, 4, 8, and 20 µL of the 500 µM standard solution to standard wells of a 96-well plate, and add the appropriate diluent to bring the volume to 20 µL. The final standard concentrations are 0, 5, 10, 20, 50, 100, 200, and 500 µM. Use a transparent 96-well plate for absorbance detection. For fluorescence detection, mix 2 µL Sarcosine Standard (5 mM) with 98 µL of the appropriate diluent to prepare a 100 µM sarcosine standard solution. Add 0, 0.4, 1, 2, 4, 10, and 20 µL of the 100 µM standard solution to standard wells of a 96-well plate, and add the appropriate diluent to bring the volume to 20 µL. The final standard concentrations are 0, 2, 5, 10, 20, 50, and 100 µM. Use a black 96-well plate for fluorescence detection. 2. Add 1–20 µL of each sample or diluted sample to sample wells, and add Lysis Buffer A for Metabolic Assay or Sarcosine Assay Buffer to bring the volume to 20 µL, using the same diluent selected for the standard curve. Include a blank control containing only the selected diluent. Record the total sample dilution factor as n; for example, if the sample was diluted 10-fold in this step and 5 µL of the diluted sample was added, n = 10 × 20/5 = 40. 3. Add 80 µL Amplex Red Working Solution to each well and mix. Incubate at 37°C protected from light for 30 minutes. If absorbance or fluorescence is weak, the reaction time may be extended to 45 or 60 minutes. 4. For absorbance detection, measure A570. For fluorescence detection, measure fluorescence intensity using an excitation wavelength of 560 nm and an emission wavelength of 590 nm. 5. Establish

	a standard curve and calculate the sarcosine concentration (A). If the sample background signal is high, subtract the background control signal from the sample signal. Absorbance detection has good linearity from 5 to 500 μM, and fluorescence detection has good linearity from 0.5 to 100 μM. Calculate the sarcosine concentration as C (μM) = A × n, where A is the sarcosine concentration determined from the standard curve and n is the total sample dilution factor. The mass concentration of sarcosine in the sample can be calculated as ng/mL = C × 89.09 based on the molecular weight of sarcosine, 89.09. Assay time Approximately 1 hour for the complete detection process; the enzymatic reaction is performed at 37°C for 30 minutes and may be extended to 45 or 60 minutes if needed. Analysis Establish a standard curve and calculate the sarcosine concentration in the sample (A). If the sample background control signal is high, subtract the sample background control signal from the sample signal. Absorbance detection has good linearity from 5 to 500 μM, and fluorescence detection has good linearity from 0.5 to 100 μM. Calculate the sarcosine concentration as C (μM) = A × n, where A is the sarcosine concentration determined from the standard curve in the final calculation step and n is the total sample dilution factor. Based on the molecular weight of sarcosine, 89.09, the mass concentration in the sample can be calculated as ng/mL = C × 89.09.
Usage	Usage For professional scientific research use only. Not for clinical diagnosis or treat ment, food, or drug use. Wear a laboratory coat and disposable gloves during operati on. Materials Required but Not Supplied PBS for washing adherent cells Heparin or E DTA for plasma preparation Centrifuge High-throughput tissue homogenizer, handhel d tissue homogenizer, or glass homogenizer for tissue sample preparation Transpare nt 96-well plate for absorbance detection Black 96-well plate for fluorescence detectio n Microplate reader capable of measuring A570 or fluorescence at 560 nm excitation/ 590 nm emission

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