

Sarcosine Assay Kit

(Catalog #K636-100; 100 assays; Store Kit at -20°C)

I. Introduction:

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. BioVision'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 ($\lambda_{max} = 570 \text{ nm}$) 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 1 – 10000 μM .

II. Kit Contents:

Components	K636-100	Cap Code	Part Number
Sarcosine Assay Buffer	25 ml	WM	K636-100-1
Sarcosine Probe (in DMSO, Anhydrous)	0.2 ml	Red	K636-100-2A
Sarcosine Enzyme mix	Lyophilized	Green	K636-100-4
Sarcosine Standard (10 μmol)	Lyophilized	Yellow	K636-100-5

III. Reagent Preparation and Storage Conditions:

- Sarcosine Assay Buffer:** Ready to use as supplied. It may be stored at 4°C or -20°C.
- Sarcosine Probe:** Briefly warm at 37°C for 1-2 min to dissolve. Mix well. Store at -20°C, protected from light and moisture. Stable for at least 2 months.
- Enzyme mix:** Reconstitute with 220 μl of Sarcosine Assay Buffer. Store at -20°C when not in use. Aliquot and store until needed. Freeze/thaw should be limited to one time.
- Sarcosine Standard:** Reconstitute with 100 μl of dH₂O to generate 100nmol/ μl Sarcosine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

IV. Assay Protocol:

- Prepare Standard:** Mix 10 μl reconstituted Sarcosine Standard with 990 μl of Assay Buffer, mix to generate 1 nmol/ μl standard working solution. Add 0, 2, 4, 6, 8, 10 μl of the working solution to 6 consecutive wells. Bring the volume to 50 μl each well Assay Buffer. If a more sensitive method is desired, fluorescence can be utilized. Further dilute the standard 10-100 fold, and follow the same procedure as for the colorimetric assay.
- Prepare Samples:** Add 0-50 μl of samples to the wells and bring the volume to 50 μl with Assay buffer. **Note:** For unknown samples, we suggest testing several different doses to ensure the readings are in the linear range of the standard curve. **Note: Urine samples do not work well with the assay due to sample interferences.**
- Prepare Reaction Mix:** Prepare enough reaction mix for the standard and samples. For each assay:
 - 46 μl Assay Buffer
 - 2 μl enzyme
 - 2 μl probe*

Mix well. Add 50 μl of the appropriate Reaction Mix to each standard and sample well, mix. Incubate at 37°C for 1 hr.

***Note:** If the background is high in fluorescence assay, 1/10 probe can be used, which will decrease background significantly.

- Read the plate in a plate reader at 570 nm, or fluorescence at Ex/Em = 538/587 nm.

V. Calculations:

- Plot standard curve:** Subtract reagent background from all readings. Plot readings vs. nmoles Sarcosine.

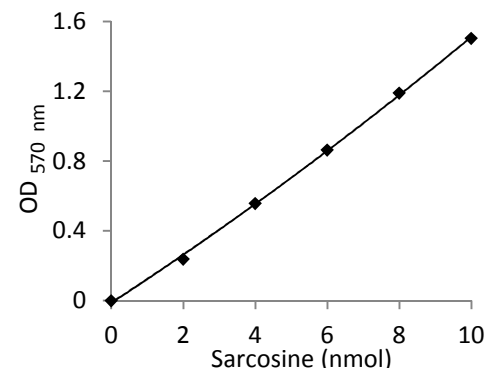
- Determine sample sarcosine concentrations:** Apply sarcosine readings to the standard curve. Sarcosine concentration:

$$C = \text{Sa}/\text{Sv} \text{ nmol}/\mu\text{l}, \text{ or mM}$$

Where Sa is the sample amount of unknown in nmol from your standard curve.

Sv is the sample volume added to the well in micro-liter.

Sarcosine Molecular Weight: 89.10.



Sarcosine Standard Curve: The assay is performed follow the kit procedure.

RELATED PRODUCTS:

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|--|-------------------------|
| Amino Acid Assay Kit | Ethanol Assay Kit |
| Creatine, Creatinine Assay Kits | Glycogen Assay Kit |
| Glycerol Assay Kit | Uric Acid Assay Kit |
| Glutamate Assay Kit | Choline Assay Kit |
| Glutathione Assay Kits | Triglyceride Assay Kits |
| Lactate, Pyruvate Assay Kits | Glycerol Assay Kits |
| Glucose, Galactose, Lactose, Sucrose Assay Kit | |

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.