# Product Information

## Sarcosine Colorimetric/Fluorometric Assay Kit

### I. Kit Contents:

| Components | K2138-100<br>100 assays | Cap Color | Part Number |
|---|---|---|---|
| Sarcosine Assay Buffer | 25 ml | WM | K2138-C-1 |
| Sarcosine Probe ( in DMSO, Anhydrous) | 0.2 ml | Red | K2138-C-2 |
| Sarcosine Enzyme mix | Lyophilized | Green | K2138-C-3 |
| Sarcosine Standard (10 µmol) | Lyophilized | Yellow | B7239 |

### II. Introduction:

Sarcosine is a natural amino acid that is present in muscles and other body tissues. Sarcosine acts as an intermediate and plays critical roles in the metabolism of methionine, glycine, serine, glutathione, choline, creatine and purine, etc. Detection of sarcosine level has wide significance in research and development.

The Sarcosine Colorimetric/Fluorometric Assay Kit provides a simple, fast and convenient way for accurate detection of sarcosine levels in various biological samples based on colorimetric and fluorometric method. In the assay, sarcosine is specifically oxidized to yield a product that converts a colorless sarcosine probe to a product with highly fluorescent (Ex/Em = 538/587 nm) and intense red color (λmax = 570 nm). Sarcosine can be easily detected with range of 1 - 10000 µM.

### III. Reagent Preparation and Storage Conditions:

1. Sarcosine Assay Buffer: Ready to use as supplied. It may be stored at 4℃ or -20℃.

2. Sarcosine Probe: Briefly warm at 37℃ for 1-2 min to dissolve. Mix well. Store at -20℃, protected from light and moisture. Stable for at least 2 months.

3. Enzyme mix: Reconstitute with 220 µl of Sarcosine Assay Buffer. Store at -20℃ when not in use. Aliquot and store until needed. Freeze/thaw should be limited to one time.

4. Sarcosine Standard: Reconstitute with 100 µl of $dH_2O$ to generate 100nmol/µl Sarcosine Standard. Dissolve completely. Store at -20℃, stable for 2 months.

### IV. Assay Protocol:

1. 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.

2. 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.

3. Prepare Reaction Mix: Prepare enough reaction mix for the standard and samples. For each assay:

| | |
|---|---|
| Assay Buffer | 46µl |
| enzyme | 2µl |

Probe 2µl

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

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

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

6. Calculations:

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

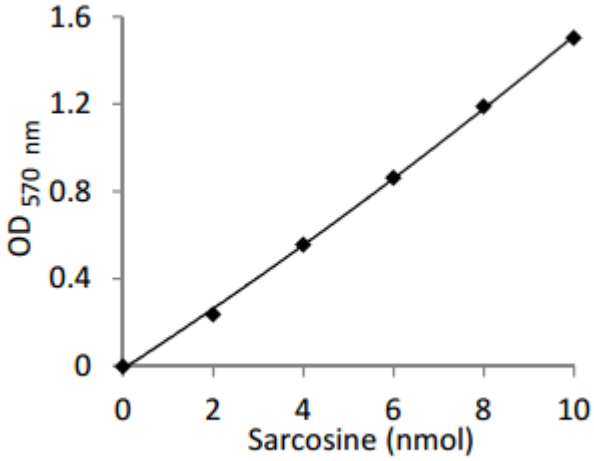
b. Determine sample sarcosine concentrations: Apply sarcosine readings to the standard curve. Sarcosine concentration:

C = Sa/Sv nmol/µl, 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-litter.

Sarcosine Molecular Weight: 89.10.




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

## General Troubleshooting Guide:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of a different buffer<br>• Omission of a step in the protocol<br>• Plate read at incorrect wavelength<br>• Use of a different 96-well plate | • Assay buffer must be at room temperature<br>• Refer and follow the data sheet precisely<br>• Check the wavelength in the data sheet and the filter settings of the instrument<br>• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type<br>• Samples prepared in a different buffer<br>• Samples were not deproteinized (if indicated in d atasheet)<br>• Cell/ tissue samples were not completely homogenized<br>• Samples used after multiple free-thaw cycles<br>• Presence of interfering substance in the sample | • Refer data sheet for details about incompatible samples<br>• Use the assay buffer provided in the kit or refer data sheet for instructions<br>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated<br>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope |

| | | |
|---|---|---|
| | • Use of old or inappropriately stored samples | • Aliquot and freeze samples if needed to use multiple times<br>• Troubleshoot if needed, deproteinize samples<br>• Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components<br>• Use of expired kit or improperly stored reagents<br>• Allowing the reagents to sit for extended times on ice<br>• Incorrect incubation times or temperatures<br>• Incorrect volumes used | • Thaw all components completely and mix gently before use<br>• Always check the expiry date and store the components appropriately<br>• Always thaw and prepare fresh reaction mix before use<br>• Refer data sheet & verify correct incubation times and temperatures<br>• Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components<br>• Pipetting errors in the standard<br>• Pipetting errors in the reaction mix<br>• Air bubbles formed in well<br>• Standard stock is at an incorrect concentration<br>• Calculation errors<br>• Substituting reagents from older kits/ lots | • Thaw and resuspend all components before preparing the reaction mix<br>• Avoid pipetting small volumes<br>• Prepare a master reaction mix whenever possible<br>• Pipette gently against the wall of the tubes<br>• Always refer the dilutions in the data sheet<br>• Recheck calculations after referring the data sheet<br>• Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength<br>• Samples contain interfering substances<br>• Use of incompatible sample type<br>• Sample readings above/below the linear range | • Check the equipment and the filter setting<br>• Troubleshoot if it interferes with the kit<br>• Refer data sheet to check if sample is compatible with the kit or optimization is needed<br>• 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. | | |

**For research use only! Not to be used in humans.**

## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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