RayBio® Sarcosine Assay Kit

User Manual Version 1.0
Mar 25, 2013

RayBio® Sarcosine Assay Kit Protocol

(Cat#:68-Sarco-S100)

RayBiotech, Inc.

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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. RayBiotech'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 (λ_{max} = 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 1 – 10000 µM.

II. KIT CONTENTS

Store Kit at –20°C

Components	Size	Cap Code	Part Number
Sarcosine Assay Buffer	25 ml	WM	Item A
Sarcosine Probe (in DMSO, Anhydrous	0.2 ml	Red	Item B
Sarcosine Enzyme mix	Lyophilized	Green	Item C
Sarcosine Standard (10 µmol)	Lyophilized	Yellow	Item D

III. REAGENTS PREPARATION AND STORAGE

- **Sarcosine Assay Buffer:** Ready to use as supplied. It may be stored at 4°C or -20°C.Substrate Mix: Reconstitute with 0.5 ml ddH2O. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.
- **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 dH_2O to generate 100nmol/µl Sarcosine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

IV. ASSAY PROCEDURE

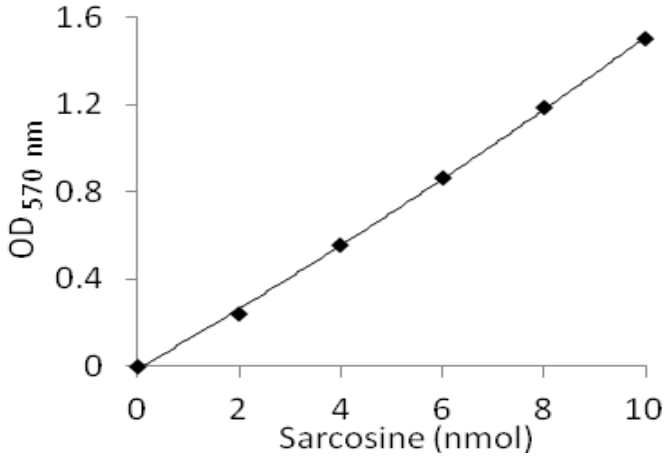
1. **Prepare Standard:** Mix 10 µl reconstituted Sarcosine Standard with 990 µl of Assay Buffer, mix to generate 1 nmol/µl standard working solution. Add 0, 2, 4, 6, 8, 10 µl of the working solution to 6 consecutive wells. Bring the volume to 50 µl each well Assay Buffer. If a more sensitive method is 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:
   46µl Assay Buffer
   2µl enzyme
   2µl probe*
4. 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.
5. Read the plate in a plate reader at 570 nm, or fluorescence at Ex/Em = 538/587 nm.

V. CALCULATIONS

1. **Plot standard curve:** Subtract reagent background from all readings. Plot readings vs. nmoles Sarcosine.
2. **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

VI. GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		

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