Product Information

Creatine Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2137-100 100 assays	Cap Color	Part Number
Creatine Assay Buffer	25 ml	WM	K2137-C-1
Creatine Probe (in DMSO)	0.2 ml	Red	K2137-C-2
Creatinase	Lyophilized	Blue	K2137-C-3
Creatine Enzyme mix	Lyophilized	Green	K2137-C-4
Creatine Standard (10 µmol)	Lyophilized	Yellow	K2137-C-5

II. Introduction:

Creatine is a nitrogenous organic acid and is naturally present in vertebrates. Creatine maintains a high ATP/ADP ratio and helps to supply energy to cells in the body, especially muscle. Creatine supplementation is used as a sport performance enhancer and to treat muscular, neurological and neurodegenerative diseases. Detection of creatine level has wide significance in research and development.

The Creatine Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for accurate detection of creatine levels in various biological fluids based on colorimetric and fluorometric method. In the assay, creatine is converted to sarcosine by creatinase. Sarcosine is then specifically oxidized to yield a product that converts a colorless probe to a highly fluorescent (Ex/Em = 538/587 nm) and intensely red color (λmax = 570 nm) product. The kit can detect 0.001 - 10 mM creatine.

III. Reconstitution of Reagents:

1. Creatine Assay Buffer: Ready to use as supplied. It may be stored at 4°C or -20°C.

2. Creatine Probe: ready to use as supplied. Warm to 18°C before use to melt frozen DMSO. Store at -20°C, protect from light and moisture. Stable for 2 months.

3. Creatinase, Creatine Enzyme mix: Reconstitute with 220 µl of Assay Buffer. Keep on ice during use. Store at -20°C when not in use. Aliquot each and store until needed. Freeze/thaw should be limited to one time.

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

IV. Assay Protocol:

1. Prepare Standard: Mix 10µl reconstituted creatine standard with 990 µl of Assay Buffer, mix to generate 1nmol/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 with Assay Buffer. If a more sensitive method is desired, fluorescence assay can be utilized. Further dilute the standard 10 - 100 fold, and follow the same procedure as for the colorimetric assay.

2. Prepare Samples: High concentrations of proteins may interfere with the assay. Samples containing proteins may be filtered through a 10kDa MW cut-off filter prior to assay. Add 0 - 50 µl of sample to the wells and bring the volume to 50 µl with Assay buffer.

Note: For unknown samples, we suggest testing several different dilutions to ensure the readings are in the linear range of the standard curve.

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

Assay Buffer	44 µl
Creatinase	2 µl

enzyme 2 µl

probe 2 µl

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: Sarcosine gives background for the assay. For samples which may contain a significant amount of sarcosine, do a background control. Prepare a reaction without the creatinase (replacing the creatinase with 2 µl assay buffer).

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

6. Calculations:

a. Plot Standard Curve: Subtract reagent background from all readings. Plot readings vs. nmoles creatine.

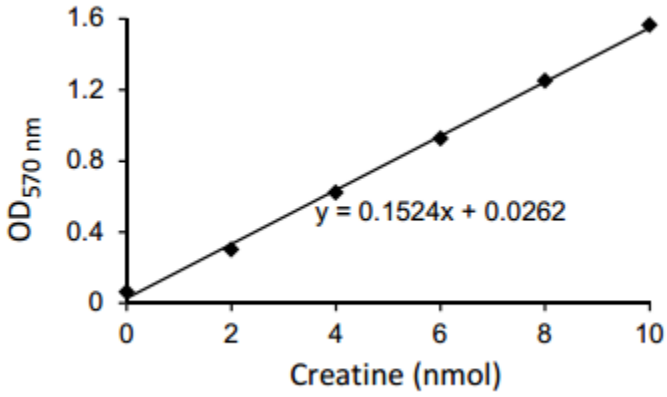
b. Determine sample Creatine concentrations: Subtract the background reading from the creatine assay sample. Apply the creatine reading to the standard curve. Creatine concentration:

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.

Creatine Molecular Weight: 131.13.



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 • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• 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 ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles	• 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);

	• Presence of interfering substance in the sample • Use of old or inappropriately stored samples	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	• 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	• 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 data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• 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	• 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	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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.		

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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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