3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Creatine Assay Kit

Catalog Number **MAK079**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Creatine is a nitrogenous compound that acts as a high-energy reservoir for the rapid regeneration of ATP. Approximately 95% of creatine is found in skeletal muscle, primarily as phosphocreatine. Creatine can be acquired through dietary consumption or formed from L-arginine, glycine, and L-methionine in a multi-step reaction that occurs in the kidneys and liver. Creatine is then transported to muscle tissue. Creatine supplementation is used for the enhancement of sports performance, primarily by increasing muscle mass. Creatine is also being investigated as a treatment of neuromuscular diseases, where it may aid in neuroprotection and by improving the cellular bioenergetic state.

In this assay, Creatine concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the Creatine present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Creatine Assay Buffer Catalog Number MAK079A	25 mL
Creatine Probe, in DMSO Catalog Number MAK079B	0.2 mL
Creatinase Catalog Number MAK079C	1 vl
Creatine Enzyme Mix Catalog Number MAK079D	1 vl
Creatine Standard, 10 µmole Catalog Number MAK079E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Creatine Assay Buffer – Allow buffer to come to room temperature before use.

Creatine Probe – Thaw at room temperature to melt solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the Creatine Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Creatine Probe Solution 5 to 10-fold with Creatine Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Creatinase and Creatine Enzyme Mix – Reconstitute each with 220 µL of Creatine Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Creatine Standard – Reconstitute in 100 µL of water to generate a 100 mM (100 nmole/µL) Creatine Standard solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Creatine Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Creatine Standard Solution with 990 µL of Creatine Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Creatine standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Creatine Assay Buffer to each well to bring the volume to 50 µL.

Creatine Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Creatine Standard Solution with 990 µL of Creatine Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Dilute 10 µL of the 1 mM standard solution with 90 µL of Creatine Assay Buffer to generate a 0.1 mM (0.1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM Creatine standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Creatine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold Creatine Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material. High concentrations of proteins may interfere with the assay and should be removed with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Serum samples may be deproteinized with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Bring samples to a final volume of 50 µL with Creatine Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Sarcosine present in the sample can generate background. To control for sarcosine background, include a blank sample for each sample by omitting the Creatinase in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Master Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
Creatine Assay Buffer	46 µL	44 µL
Creatinase	–	2 µL
Creatine Enzyme Mix	2 µL	2 µL
Creatine Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Creatine Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Creatine standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Creatine present in the sample may be determined from the standard curve

Concentration of Creatine

$$S_a/S_v = C$$

S_a = Amount of Creatine in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Creatine in sample

Creatine molecular weight: 131.13 g/mole

Sample Calculation

Amount of Creatine (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Creatine in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 131.13 \text{ ng/nmole} = 15.32 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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