



Creatine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1185

(Version 1.4B)

Detection and Quantification of Creatine Content in Urine, Serum,
Plasma, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Creatine is present in vertebrates and helps to supply energy to muscle. In humans and animals, approximately half of creatine originates from food (mainly from fresh meat). Creatine supplementation has been investigated as a possible therapeutic approach for the treatment of muscular, neuromuscular, neurological and neurodegenerative diseases.

Creatine Colorimetric Microplate Assay Kit provides an accurate, convenient measure of creatine concentration in biological fluids such as serum, urine or CSF. In the assay, creatine is converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color, can be measured at a colorimetric readout at 546 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	20mlx 1	4 °C
Enzyme	Powderx 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 546 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Enzyme: Add 9 ml Reaction Buffer to dissolve before use. Store at 4 °C for 4 hours.

Dye Reagent: Add 10 ml Reaction Buffer to dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 100 mmol/L of standard stock solution, store at 4 °C for 1-2 months after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 50 μ l stock solution into 950 μ l distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

V. SAMPLE PREPARATION

1. For urine, serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Standard**	Blank	Sample
Standard	10 µl	--	--
Distilled water	--	10 µl	--
Sample	--	--	10 µl
Enzyme	90 µl	90 µl	90 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it in the oven, 37 °C for 30 minutes, measured at 546 nm and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

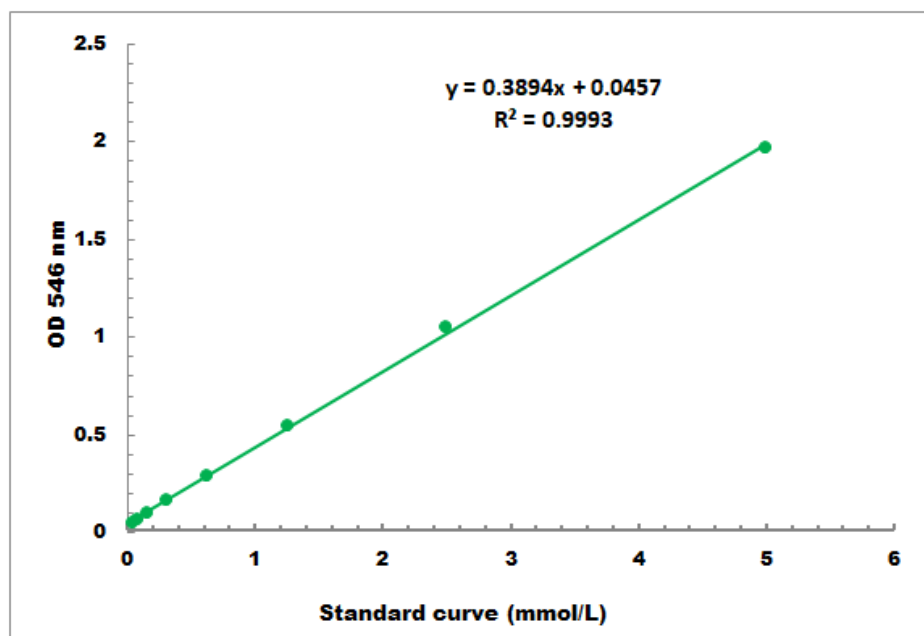
C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L