Creatine Microplate Assay Kit

User Manual

Catalog # CAK1185

(Version 1.3B)

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

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

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 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	20 ml x 1	4 °C
Enzyme	Powder x 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	

Note:

Enzyme: add 9 ml Reaction Buffer to dissolve before use.

Dye Reagent: add 10 ml Reaction Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 50 µl into 950 µl distilled water. The concentration will be 5 mmol/L.

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. SAMPLE PREPARATION

1. For urine, serum or plasma samples

Detect directly.

V. 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

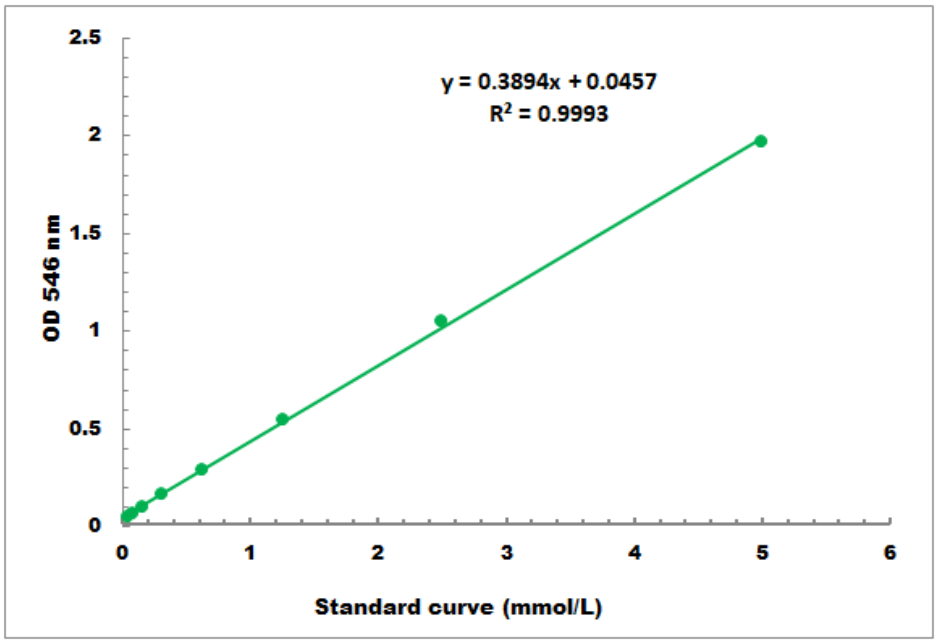
$$\text{Creatine (mmol/L)} = (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times C_{Standard}$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 5 mmol/L.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES