



# **Creatinine Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1220**

(Version 1.3A)

Detection and Quantification of Creatinine 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

Creatinine is synthesized in the body at a fairly constant rate from creatine, which is produced during muscle contractions from creatine phosphate. In the blood, creatinine is removed by filtration through the glomeruli of the kidney and is secreted into urine. In healthy individuals, creatinine secretion is independent of diet and is fairly constant. The creatinine clearance test has become one of the most sensitive tests for measuring glomerular filtration rate. In kidney disease, creatinine levels in the blood are elevated, whereas the creatinine clearance rate and hence the urine levels are diminished. Creatinine test is most widely used to assess kidney function. Simple, direct and automation-ready procedures for measuring creatinine concentration in biological samples are becoming popular in Research and Drug Discovery.

Creatinine Microplate Assay Kit provides an accurate, convenient measure of creatinine concentration in biological fluids such as serum, urine or CSF. In the assay, creatinine is converted to creatine by creatininase, 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 $\mu$ l	--	--
Distilled water	--	10 $\mu$ l	--
Sample	--	--	10 $\mu$ l
Enzyme	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l
Dye Reagent	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
Mix, put it in the oven, 37 °C for 15 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

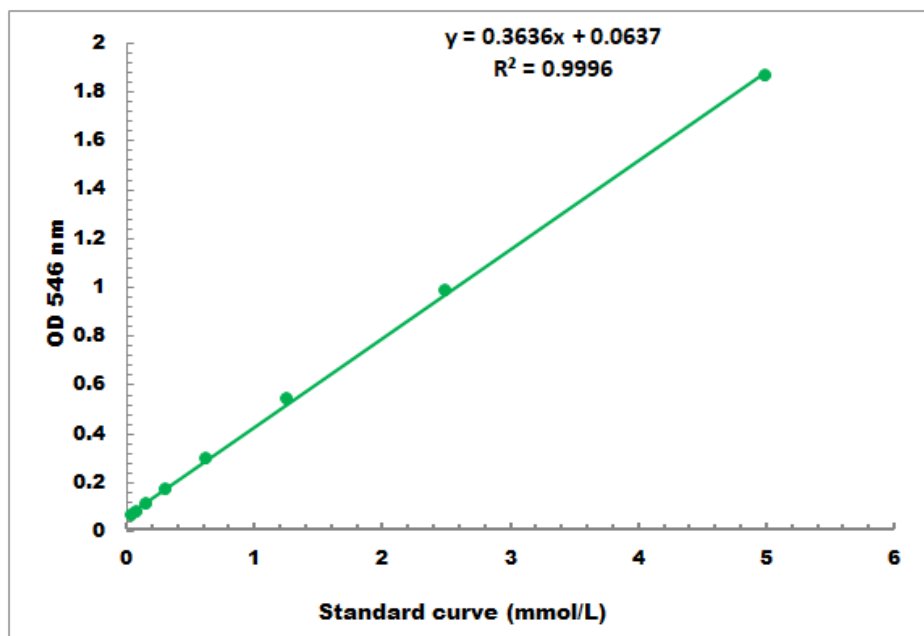
1. According to the volume of sample

$$\begin{aligned}\text{Creatinine (mmol/L)} &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times C_{\text{Standard}} \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

$C_{\text{Standard}}$ : 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](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES