Urea Microplate Assay Kit

User Manual

Catalog # CAK1160

(Version 1.5D)

Detection and Quantification of Urea Content in Urine, Serum, Tissue extracts, Cell lysate, Cell culture, Other biological fluids media Samples.

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

I. INTRODUCTION

Urea is primarily produced in the liver and secreted by the kidneys. Urea is the major end product of protein catabolism in animals. It is the primary vehicle for removal of toxic ammonia from the body. Urea determination is very useful for the medical clinician to assess kidney function of patients. In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extrarenal diseases, e.g., congestive heart failure, liver diseases and diabetes. Decreased levels indicate acute hepatic insufficiency or may result from overvigorous parenteral fluid therapy.

Urea Microplate Assay Kit is designed to measure urea directly in biological samples without any pretreatment. The intensity of the color, measured at 620nm, is directly proportional to the urea concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Enzyme	Powder x 1	-20 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	3 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer into Enzyme tube to dissolve before use.

Dye Reagent I: add 7 ml distilled water to dissolve before use.

Dye Reagent II: add 3 ml Dye Reagent II Diluent into Dye Reagent II, mix before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.05 ml into 0.95 ml distilled water, mix, the concentration will be 250 mg/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulation oven

IV. SAMPLE PREPARATION

1. For urine, serum or other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Shake and mix, put it into the oven, 37 °C for 10 minutes.			
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl
Shake and mix, put it into the oven, 37 °C for 30 minutes. Then record absorbance measured at 620 nm.			

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

$$\text{Urea (mg/L)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 250 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

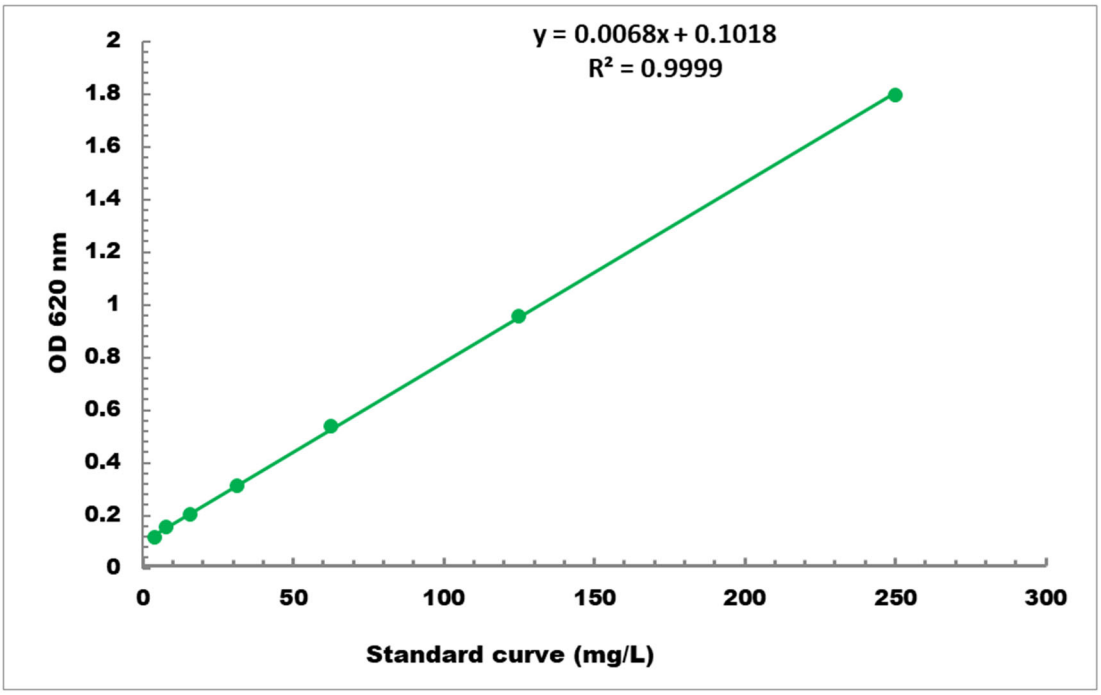
$C_{Standard}$: the standard concentration, 250 mg/L;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml.

VII. TYPICAL DATA

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



Detection Range: 5 mg/L - 250 mg/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