Instruction - Urea Microplate Assay Kit

Cat. No.: ARS6755

Detection and Quantification of Urea Content in Urine, Serum, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids 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 Colorimetric Microplate Assay Kit is designed to measure urea directly in biological samples without any pretreatment. The intensity of the color, measured at 620 nm, 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	9 ml x 1	4 °C
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 2	4 °C, keep in dark
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	

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. REAGENT PREPARATION

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer into Enzyme tube to dissolve before use. Store at 4 °C for 2 days or -20 °C for 2 weeks after reconstitution.

Dye Reagent I: Add 7 ml distilled water to dissolve, mix thoroughly before use. Keep in dark and store at 4 °C for 3 days.

Dye Reagent II: Add 1.5 ml Dye Reagent II Diluent into each Dye Reagent II vial, mix before use. Keep in dark and store at 4 °C for 3 days.

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 5000 mg/L of standard stock solution, store at 4 °C for 1 month after reconstitution. Then dilute to 200 mg/L standard top solution by adding 0.04 ml stock solution into 0.96 ml 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 200/100/50/25/12.5/6.25/3.12 mg/L.

V. SAMPLE PREPARATION

For urine, serum or other biological fluids samples:
Dilute with distilled water or detect directly.

VI. 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, incubate at 37°C for 15 minutes.			
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl
Shake and mix, incubate at 37°C for 10 minutes. Then record absorbance at 620 nm.			

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 according to the slope of the standard curve:

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

Calculate the initial concentration according to sample preparation procedure.

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

$$C = \frac{(C_{standard} \times V_{standard}) \times (OD_{sample} - OD_{blank})}{(OD_{standard} - OD_{Blank}) \times V_{Sample}} \ (mg/L)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

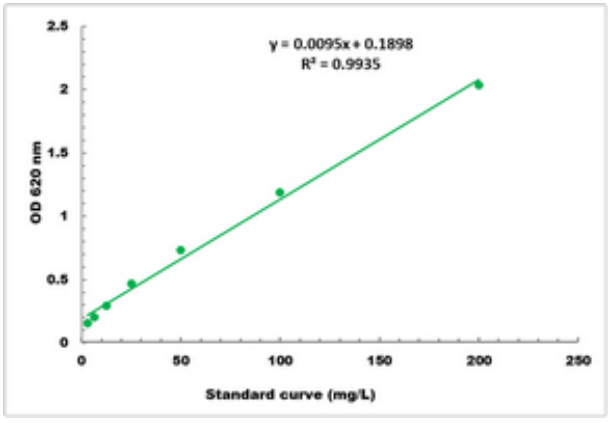
$C_{standard}$: the standard concentration, mg/L

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

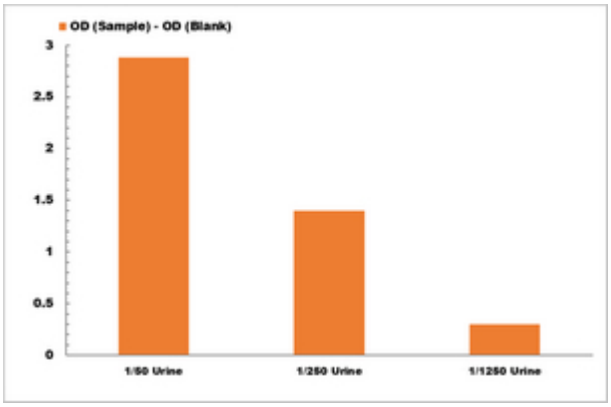
V_{Sample}: the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

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



Detection Range: 2 mg/L – 200 mg/L



Determination of urea in urine

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