



Urease Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1180

(Version 1.5D)

Detection and Quantification of Urease activity in Urine, Tissue
extracts, Cell lysate, Cell culture, Other biological fluids Samples.

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

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I. INTRODUCTION

Urease (Amidohydrolase, EC 3.5.1.5) is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Many gastrointestinal or urinary tract pathogens produce urease. Thus its activity is a useful diagnostic parameter for the presence of pathogens such as *Helicobacter pylori*. Urease is found in bacteria, yeast, and higher plants. Urease activity is commonly determined in anaerobes of the bovine rumen, human feces and environmental samples such as soils and phytoplanktons.

Urease Activity Colorimetric Microplate Assay Kit provides a very sensitive and convenient means to measure urease activity in a variety of samples. In the assay, urease reacts with urea, resulting in the formation of ammonia, which is determined at 620nm, is directly proportional to the urease activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	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	5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °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

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 200 mmol/L of standard stock solution, store at 4 °C for 5-6 months after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 25 μ l stock solution into 975 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.

Substrate: Add 10 ml Substrate Diluent to dissolve before use. Store at 4 °C for 5-6 months after reconstitution.

Dye Reagent I: Add 4 ml distilled water to dissolve before use. Keep in dark for immediate use after reconstitution.

Dye Reagent II: Add 5 ml Dye Reagent II Diluent into Dye Reagent II, mix before use. Keep in dark and store at 4 °C for 1-2 weeks after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For urine and other biological fluids media samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Substrate	100 μ l	100 μ l	--	--	100 μ l
Sample	10 μ l	--	--	--	--
Positive Control	--	--	--	--	10 μ l
Standard	--	--	10 μ l	--	--
Distilled water	--	10 μ l	100 μ l	110 μ l	--
Shake and mix, put it into the oven, 37°C for 10 minutes.					
Dye Reagent I	40 μ l	40 μ l	40 μ l	40 μ l	40 μ l
Dye Reagent II	50 μ l	50 μ l	50 μ l	50 μ l	50 μ l
Shake and mix, put it into the oven, 70°C for 5 minutes, then record absorbance measured at 620 nm.					

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One unit of Urease activity is defined as the enzyme produces 1 μ mol of ammonia per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

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

2.1 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

2.4 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

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

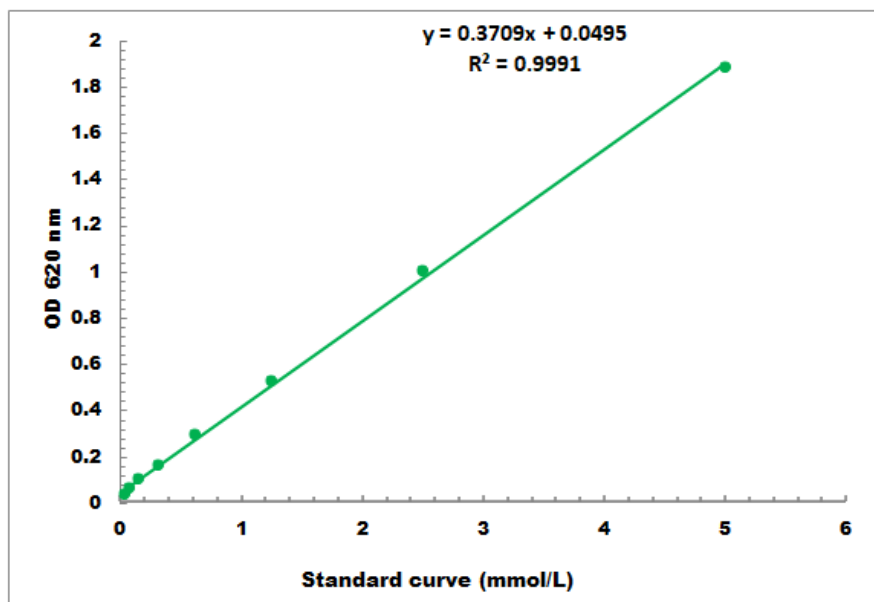
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

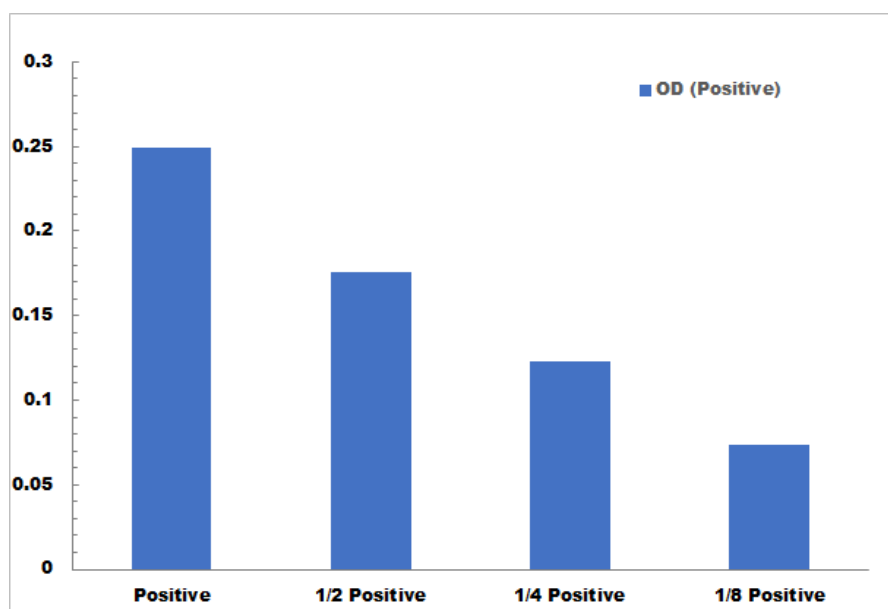
T: the reaction time, minute

VIII. TYPICAL DATA

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



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



Positive Control reaction in 96-well plate assay with decreasing the concentration