# Urease Microplate Assay Kit

# User Manual

## Catalog # CAK1180

(Version 1.4D)

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.**

## 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 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 ml x 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 | |

**Note:**

**Substrate**: add 10 ml Substrate Diluent to dissolve before use.

**Dye Reagent I**: add 4 ml distilled water to dissolve before use.

**Dye Reagent II**: add 5 ml Dye Reagent II Diluent into Dye Reagent II, mix before use.

**Standard**: add 1 ml distilled water to dissolve, then add 25 µl to 975 µl distilled water, mix, the concentration will be 5 mmol/L.

**Positive Control**: add 1 ml Assay Buffer to dissolve before use.

## 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. 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 $5\times10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 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 10000g 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.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent | Sample | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Substrate | 100 µl | 100 µl | -- | -- | 100 µl |
| Sample | 10 µl | -- | -- | -- | -- |
| Positive Control | -- | -- | -- | -- | 10 µl |
| Standard | -- | -- | 10 µl | -- | -- |
| Distilled water | -- | 10 µl | 100 µl | 110 µl | -- |
| Shake and mix, put it into the oven, 37 °C for 10 minutes. | | | | | |
| Dye Reagent I | 40 µl | 40 µl | 40 µl | 40 µl | 40 µl |
| Dye Reagent II | 50 µl | 50 µl | 50 µl | 50 µl | 50 µl |
| Shake and mix, put it into the oven, 70 °C for 5 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) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

## VI. CALCULATION

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

1. According to the volume of liquid sample

$$\text{Urease (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{Urease (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × $10^4$;

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

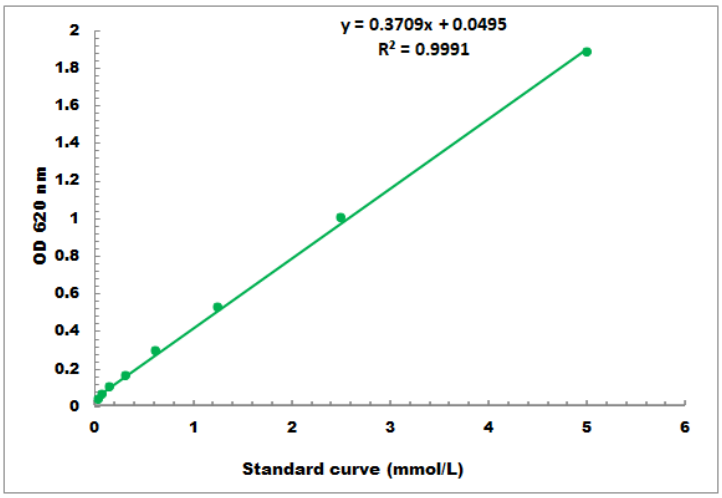
$V_{Sample}$: the volume of sample, 0.01 ml;

$V_{Assay}$: the volume of Assay buffer, 1 ml;

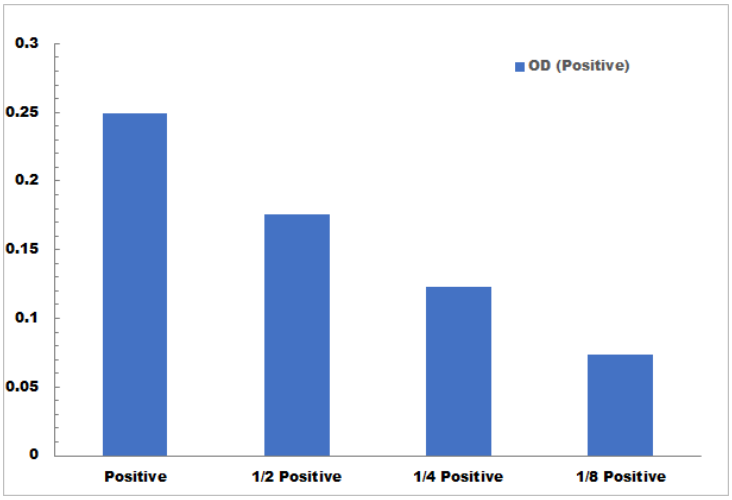
T: the reaction time, 10 minutes.

## 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




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

## VIII. TECHNICAL SUPPORT

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

## IX. NOTES