Urease Assay Kit

Catalog No.: abx298904

Size: 100 Assays

Storage: Store the positive control at -20°C and all other kit components at 4°C.

Application: For quantitative detection of Urease activity in urine, tissue homogenates, cell lysates, urine, and other biological fluids.

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

Introduction: Urease is an enzyme responsible for the hydrolysis of urea into carbon dioxide and ammonia. Many pathogens of the digestive system (such as *H. pylori*) produce urease, and so urease activity can be used as an indirect measure of pathogen activity. Urease is a common enzyme found across bacteria, plants and yeast.

Urease reacts with Urea in the assay to form ammonia. The concentration of the ammonia product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 620 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Dye Reagent A: 1 vial
4. Dye Reagent B Solution: 5 ml
5. Standard: 1 vial
6. Substrate: 1 vial
7. Substrate diluent: 10 ml
8. Positive Control: 1 vial
9. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (620 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Orbital shaker
8. Oven
9. Timer
10. Ice
11. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 10 ml of Substrate Diluent into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent A Solution**
  Add 4 ml of distilled water to the Dye Reagent A vial and mix thoroughly.

- **Standard Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly. Add 25 µl of this solution to 975 µl of distilled water to prepare a 1 ml Standard Solution with concentration 5 mmol/L.

- **Positive Control Solution**
  Add 1 ml of Assay Buffer to the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice, then allow to stand for 2 hours. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

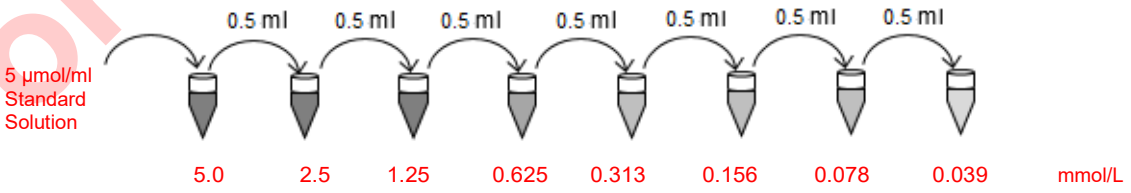
- **Urine and other biological fluids**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 5 mmol/L, 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.313 mmol/L, 0.156 mmol/L, 0.078 mmol/L, and 0.039 mmol/L.
   Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L standard solution to the 1st tube (2.5 mmol/L) and mix thoroughly.
   Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, control, standard, positive control and blank wells. We recommend setting up each standard and sample in duplicate.
3. Add 100 µl of Substrate Solution to the sample, control and positive control wells.
4. Add 10 µl of sample to the sample wells.
5. Add 10 µl of sample to the positive control wells.
6. Add 10 µl of each pre-prepared standard to the corresponding standard wells.
7. Add 10 µl of distilled water to the control wells.
8. Add 100 µl of distilled water to the standard wells.
9. Add 110 µl of distilled water to the blank wells.
10. Mix the plate using an orbital shaker. Incubate at 37°C for 10 minutes.

11. Add 40 µl of Dye Reagent A Solution to all wells.
12. Add 50 µl of Dye Reagent B Solution to all wells.
13. Mix the plate using an orbital shaker. Incubate at 37°C for 15 minutes.
14. Read and record absorbance at 620 nm.

C. Calculations

One unit of Urease activity (U) is defined as the amount of enzyme required to produce 1 µmol of ammonia per minute.

Urease activity per g of sample:

$$\textbf{Pepsin (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Urease activity per 10^4 cells or bacteria:

$$\textbf{Pepsin (U/10}^{\mathbf{4}}\textbf{ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Urease activity per ml of sample:

$$\textbf{Pepsin (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{0.5} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$	Concentration of highest standard (5 mmol/L)
T	Reaction time (10 minutes)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.01 ml)