# Urease Activity Assay Kit

Urease Activity Assay Kit can be used to measure Urease Activity in biological and environmental samples.

Catalog number: ARG82196

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## INTRODUCTION

Ureases (EC 3.5.1.5), functionally, belong to the superfamily of amido-hydrolases and phosphor-triesterases. Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates, as well as in soils, as a soil enzyme. They are nickel-containing metalloenzymes of high molecular weight.

These enzymes catalyze the hydrolysis of urea into carbon dioxide and ammonia:

$(NH2)2CO + H2O \rightarrow CO2 + 2NH3$

The hydrolysis of urea occurs in two stages. In the first stage, ammonia and carbamate are produced. The carbamate spontaneously and rapidly hydrolyzes to ammonia and carbonic acid. Urease activity increases the pH of its environment as ammonia is produced, which is basic. [Wikipedia: Urease]

## PRINCIPLE OF THE ASSAY

This Urease Activity Assay Kit provides a simple and convenient procedure for measuring Urease Activity in samples. In the assay, urease reacts with urea, resulting in the formation of ammonia, which is determined by the Berthelot method at 670nm. The activity of Urease in the sample is then determined by comparing the signals of samples to the standard.

## MATERIALS PROVIDED & STORAGE INFORMATION

| Component | Quantity | Storage information |
|---|---|---|
| Assay Buffer | 20 ml (Ready to use) | 4°C |
| Urea | 1.5 ml | 4°C |
| Reagent A | 12 ml (Ready to use) | 4°C |
| Reagent B | 6 ml (Ready to use) | 4°C |
| $NH_4Cl$ (50 mM) | 100 µl | 4°C |

The kit is shipped on ice. Store all components at 4°C in dark. Shelf life of six months after receipt.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 670 nm.
- Flat bottomed 96-well microplate
- Pipettes and pipette tips

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Prior to assay, concentrations of protein, inhibitor, substrate and incubation time may need to be established for a given sample.
- Briefly spin down the reagents before use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Ammonia is known to interfere with this assay and prior to assay, should be removed by dialysis or filtration.

- All reagents should be warmed to room temperature before use. During experiment, keep thawed Enzyme in a refrigerator or on ice.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Note: ammonia is known to interfere with this assay and prior to assay, should be removed by dialysis or filtration.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot & store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or -80°C. Avoid repeated freeze-thaw cycles.

Soil and other environmental samples - Soil and other environmental samples can be extracted in Assay Buffer (10 mM sodium phosphate, pH 7.0). Clear supernatant containing urease was obtained by centrifugation for 5 min at 14,000 g. Or any established methods can also be used. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or -80°C. Avoid repeated freeze-thaw cycles.

Urine or Cell Culture Supernatants- Remove particulates by centrifugation. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or-80°C. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

- Standard:

  - For Standard curve, prepare a 500 µM $NH_4Cl$ premix by adding 5 µL of 50 mM $NH_4Cl$ stock into 495 µL of Assay Buffer, mix well.

  - Dilute $NH_4Cl$ as follows table:

  The example of the dilution of standards

| Standard No. | Assay Buffer (µl) | 500 µM $NH_4Cl$ (µl) | $NH_4^+$ Conc. µM |
|---|---|---|---|
| S1 | 0 | 100 | 500 |
| S2 | 20 | 80 | 400 |
| S3 | 40 | 60 | 300 |
| S4 | 60 | 40 | 200 |
| S5 | 70 | 30 | 150 |
| S6 | 80 | 20 | 100 |
| S7 | 90 | 10 | 50 |
| S0 | 100 | 0 | 0 |

- Sample: If the initial assay found samples contain Urease Activity higher than25 U/L, the samples can be diluted with Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

  (It is recommended to do pre-test with different dilutions to determine the suitable dilution factor).

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use.

1. Add 90 µL of standard (S0-S7) and (diluted) samples to separate wells in a 96 well plate. The S0 is Assay buffer only serve as a Sample Blank.
2. Add 10 µL of the Urea to each well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 10 min at 30 or 37°C. (Note: For low urease activity samples, such as soil samples, incubate the urease reaction for 2 to 4 hours at 30 or 37°C)
4. Add 100 µL of the Reagent A to each well. Gently tap the plate to ensure thorough mixing.
5. Add 50 µL of the Reagent B to each well. Gently tap the plate again to ensure thorough mixing.
6. Incubate for 30 min at 37°C in dark.
7. Read the OD with a microplate reader at 670 nm (630-700 nm) immediately.

Note: For samples may contain very low concentrations of ammonia, such as soil, prepare each Sample Blank by mixing the following in this order:
100 µL Reagent A, 90 µL Sample, 10 µL Urea and 50 µL Reagent B. immediately prior to step 4. (Reagent A terminates the urease reaction) Then proceed directly to step 6.

Summary:

<table>
<tr><td></td><td>Assayed sample</td><td>Standard S0-S7</td><td>(Optional) Sample blank (for ammonia containing samples</td></tr>
<tr><td>Sample</td><td>90 µl</td><td>-</td><td>-</td></tr>
<tr><td>Standards</td><td>-</td><td>90 µl</td><td>-</td></tr>
<tr><td>Urea</td><td>10 µl</td><td>10 µl</td><td>-</td></tr>
<tr><td colspan="3">Mix well, Incubate for 10 min at RT.<br>(For soil samples, incubate for 2 to 4 hours at 30 or 37°C)</td><td>-</td></tr>
<tr><td>Reagent A</td><td>100 µl</td><td>100 µl</td><td rowspan="3">Reagent mixture:<br>100 µL Reagent A,<br>90 µL Sample,<br>10 µL Urea<br>50 µL Reagent B</td></tr>
<tr><td colspan="3">Mix well.</td></tr>
<tr><td>Reagent B</td><td>50 µl</td><td>50 µl</td></tr>
<tr><td colspan="4">Mix well and incubate for 30 min at RT in dark.</td></tr>
<tr><td colspan="4">Read the OD with a microplate reader at 670 nm immediately.</td></tr>
</table>

## CALCULATION OF RESULTS

1. Determine the slope and calculate the Urease concentration of the Samples as follows:

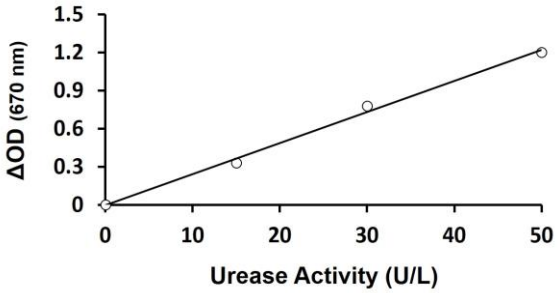
   [Urease Activity] (U/L)=

   N X [(OD $_{SAMPLE}$ – OD $_{BLANK}$) / (Slope X t]]

2. The **OD $_{Sample}$** and **OD $_{Blank}$** are OD readings of the Sample, and sample blank (S0). **N** is the sample dilution factor. **t** is the incubation time (10 min) for standard urease assay.
3. If the calculated Urease concentration is >25 U/L, dilute sample in assay buffer and repeat assay. Multiply result by the dilution factor N.
4. Unit definition: one unit of urease catalyzes the formation of 1 µmole ammonia per min at pH 7.0 under the assay conditions.
5. For example: a 0.5 g soil sample was homogenized in 10 mL of Assay Buffer (10 mM sodium phosphate, pH 7.0) (50 g soil /L). Clear supernatant containing urease was obtained by centrifugation for 5 min at 14,000 g. Enzyme reaction was performed according to the protocol for 4 hours at 30 °C.

   At the end of the reaction, 39.5 µM ammonium was determined, which corresponds to a urease activity of 39.5 µmoles/L ÷ 240 min (reaction time) = 0.165 U/L.
   Or 39.5 µmoles/L ÷ (50 g/L x 4 hours) = 0.1975 µmoles per gram per hour.

## EXAMPLE OF ASSAY




## QUALITY ASSURANCE

### Sensitivity

Linear detection range:

Colorimetric assays: 0.003 U/L to 25 U/L

The minimum detectable dose (MDD) of Urease was:

Colorimetric assays: 0.003 U/L