

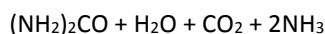
Urease Assay Kit (Colorimetric)

LS-K209-100 (100 Tests) • Store at 4°C



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 phytoplankton. This Urease Assay Kit provides a very sensitive and convenient means to measure urease activity in a variety of samples including soil. In the assay, urease reacts with urea, resulting in the formation of ammonia, which is determined by the Berthelot Method at 670nm. The assay is simple, sensitive, stable and high-throughput adaptable.

Key Features

- Safe. Non-radioactive assay.
- Sensitive and accurate. As low as 0.003 U/L urease activity can be quantified.
- Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.
- Robust and amenable to HTS: can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Urease activity determination in biological and environmental samples. Evaluation and screening for urease inhibitors.

Components

Component	K209-100
	100 Tests
Assay Buffer (pH 7.0)	20 mL
Reagent A	12 mL
Reagent B	6 mL
Urea	1.5 mL
NH ₄ Cl (50 mM)	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plate (e.g. Corning Costar).

Storage

The kit is shipped at room temperature. Store all reagent at 4°C. This product is shipped at room temperature. Shelf life of 6 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Procedure

Interference: ammonia is known to interfere with this assay and prior to assay, should be removed by dialysis or filtration.

1. Assay Preparation. Prior to assay, bring all components to room temperature. For calibration curve, prepare a 500 μM premix by mixing 5 μL 50mM NH_4Cl and 495 μL Buffer. Dilute NH_4Cl as follows

No	Premix + Buffer	Vol (μL)	NH_4^+ (μM)
1	100 μL + 0 μL	100	500
2	80 μL + 20 μL	100	400
3	60 μL + 40 μL	100	300
4	40 μL + 60 μL	100	200
5	30 μL + 70 μL	100	150
6	20 μL + 80 μL	100	100
7	10 μL + 90 μL	100	50
8	0 μL + 100 μL	100	0

Transfer 90 μL into separate wells of a clear flat-bottom 96-well plate.

Samples. Dilute sample in Assay Buffer (Note: it is prudent to test different dilutions to ensure urease activity is within the detection range). Transfer 90 μL sample into separate wells. Use 90 μL enzyme buffer as a Sample Blank.

2. Enzyme Reaction. Add 10 μL Urea to each well. Incubate at desired temperature for 10 min.
3. Detection. Add 100 μL Reagent A to each well. Tap plate to mix. Then add 50 μL Reagent B to each well. Tap plate to mix again. Note: addition of Reagent A terminates the urease reaction.

Incubate for 30 min in the dark. Read optical intensity at 670nm (630-700nm).

Calculations

Plot NH_4Cl calibration curve and determine its Slope (μM^{-1}). Urease enzyme activity in the sample is calculated as

$$\text{Urease Activity} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope} \times t} \quad (\text{U/L})$$

where $\text{OD}_{\text{SAMPLE}}$ and OD_{BLANK} are the measured OD values of the Sample and Sample Blank (enzyme buffer). t is the incubation time (10 min) for standard urease assay. If urease activity is higher than 25 U/L, dilute enzyme in assay buffer, repeat assay and multiply the calculated activity by the dilution factor.

Unit definition: one unit of urease catalyzes the formation of 1 μmole ammonia per min at pH 7.0 under the assay conditions.

General Considerations

1. Soil and other environmental samples can be extracted in Assay Buffer (10 mM sodium phosphate, pH 7.0) using any established methods. For such low urease activity samples, incubate the urease reaction for 2 to 4 hours at 30 or 37°C (Step 2).

Soil samples may contain very low concentrations of ammonia. To correct for sample ammonia, immediately prior to detection (Step 3), prepare Sample Blank by mixing the following in this order: 100 μL Reagent A, 90 μL sample extract, 10 μL urea and 50 μL reagent B.

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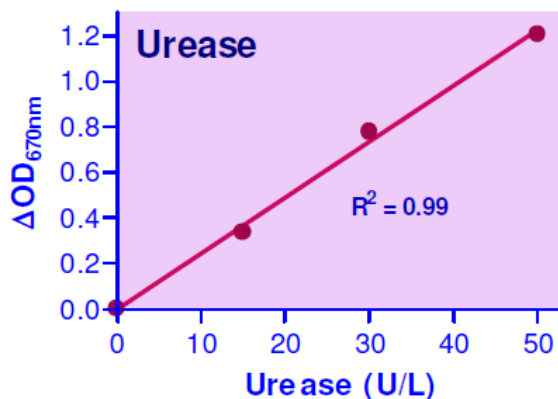


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2. Cuvette assays: scale up 4-fold to a total of 1 mL reaction by using 360 μ L Sample, 40 μ L Urea, 400 μ L Reagent A and 200 μ L Reagent B.

Sample Data

A 0.5 g soil sample was homogenized in 10 mL 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, 29.4 μ M ammonium was determined, which corresponds to a urease activity of 29.4 μ moles/L $\div$ 240 min = 0.123 U/L, or 29.4 μ moles/L $\div$ (50 g/L x 4 hours) = 0.15 μ moles per gram per hour.



96-well assay with purified urease

Version: V.08.09.2018

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