QuantiChrom™ Urease Assay Kit (DURE-100)

Quantitative Determination of Urease Activity

DESCRIPTION

UREASE (Amidohydrolase, EC 3.5.1.5) is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia.

$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$$

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.

BioAssay Systems' 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.

KIT CONTENTS

Assay Buffer:	20 mL (pH 7.0)	**Reagent A:**	12 mL
Urea:	1.5 mL	**Reagent B:**	6 mL
NH_4Cl:	100 µL 50 mM		

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY 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	100µL + 0µL	100	500
2	80µL + 20µL	100	400
3	60µL + 40µL	100	300
4	40µL + 60µL	100	200
5	30µL + 70µL	100	150
6	20µL + 80µL	100	100
7	10µL + 90µL	100	50
8	0µL + 100µL	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).

CALCULATION

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

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

where OD_{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*).

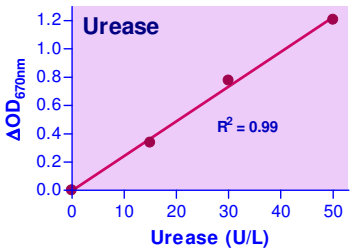
Soil samples may contain very low concentrations of ammonia. To correct for sample ammonia, immediately prior to detection (*Step 3*), prepare Sample Blank by mixing the following in this order: 100 µL Reagent A, 90 µL sample extract, 10 µL urea and 50 µL reagent B.

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

MATERIALS REQUIRED, BUT NOT PROVIDED

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

96-well assay with purified urease



Example: 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 µM ammonium was determined, which corresponds to a urease activity of 29.4 µmoles/L ÷ 240 min = 0.123 U/L, or 29.4 µmoles/L ÷ (50 g/L x 4 hours) = 0.15 µmoles per gram per hour.

PUBLICATIONS

1. Liu, Q. W. et al. (2018). Therapeutic efficiency of human amniotic epithelial stem cell-derived functional hepatocyte-like cells in mice with acute hepatic failure. Stem cell research & therapy, 9(1), 321.

2. Wu, D (2015). Constitutive expression of the DUR1, 2 gene in an industrial yeast strain to minimize ethyl carbamate production during Chinese rice wine fermentation. FEMS Microbiology Letters 363(1): fnv214.

3. Zhang, T. et al (2015). Development of an Agrobacterium-mediated transformation system for the cold-adapted fungi Pseudogymnoascus destructans and P. pannorum. Fungal Genetics and Biology 81: 73-81.