



# Urease Activity Assay Kit (Colorimetric)

rev 08/15

(Catalog # K378-100; 100 assays; Store at -20°C)

## I. Introduction:

Urease (EC 3.5.1.5) catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Urease is found in certain bacteria, yeast, fungi, and plants. In addition, it is found environmentally in soil samples and fecal matter. Due to ammonia production, urease can play a role in the pathogenesis of certain medical conditions including urinary stones, peptic ulcers, and hepatic encephalopathy. As such, urease can be used as a diagnostic for gastrointestinal and urinary tract pathogens. BioVision's Urease Activity Assay Kit provides a quick and easy way to determine urease activity. The kit uses a modified Berthelot method to detect ammonia, which can be measured at 670 nm. The kit is suitable for measuring urease activity of biological and soil samples as well as of purified enzyme. The limit of detection is below 0.001 mU.

## II. Application:

- Measurement of urease activity

## III. Sample Type:

- Purified enzyme
- Biological samples such as cells, feces, urine etc.
- Soil samples

## IV. Kit Contents:

Components	K378-100	Cap Code	Part Number
Urease Assay Buffer	15 ml	NM	K378-100-1
Ammonia Reagent 1	8 ml	Amber	K378-100-2
Ammonia Reagent 2	4 ml	Clear	K378-100-3
Urea (50x)	1.5 ml	Red	K378-100-4
Urease	1 vial	Blue	K378-100-5
Ammonium Chloride Standard (100 mM)	0.1 ml	Yellow	K378-100-6

## V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- 50 mM sodium acetate, pH 5 (for soil sample preparation only)

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Urease Assay Buffer:** Store at 4°C or -20°C. Bring to room temperature before use.
- **Ammonia Reagent 1, Ammonia Reagent 2, and Ammonium Chloride Standard:** Bring to room temperature before use.
- **Urea (50x):** Make 1x by diluting with dH<sub>2</sub>O. Bring to room temperature before use.
- **Urease:** Dissolve in 220 µl Urease Assay Buffer. Aliquot and store at -20°C. Stable for two months. Keep on ice during use.

## VII. Urease Activity Assay Protocol:

1. **Sample Preparation:** Homogenize cells (2 x 10<sup>6</sup> cells/ml) in appropriate buffer (e.g. PBS) with protease inhibitors (Cat. # K271 or equivalent). Centrifuge at 10,000xg for 10 min. at 4°C. Collect supernatant. For recombinant or purified enzyme, dissolve in Urease Assay Buffer. Add 1-10 µl sample or enzyme into desired wells in a 96-well plate. For positive control, dilute Urease 1:10 by adding 10 µl urease into 90 µl Urease Assay Buffer. Add 2-10 µl of diluted Urease into desired well. Adjust the volume of positive control and sample wells to 10 µl per well with dH<sub>2</sub>O. For reagent background control, add 10 µl Urease Assay Buffer.

**Soil Sample Preparation:** Homogenize 0.5 g soil sample in 10 ml of 50 mM sodium acetate, pH 5 for 2 min. Stir vigorously and add 0.9 ml into a clean tube as test sample and 0.2 ml into another tube as soil background control. Centrifuge soil background control tube at 8000 g for 1 min. and collect 0.1 ml supernatant. Add supernatant into desired well in a 96-well plate. Proceed directly to step 4 (develop and measure) immediately. To test sample, add 0.1 ml of 1x Urea and incubate at 37°C for 2 hrs. Centrifuge at 8000 g for 1 min. Collect 0.1 ml supernatant and add into desired well in a 96-well plate. Proceed directly to step 4.

### Notes:

- For samples with unknown urease activity, we suggest testing several amounts of enzyme or cell homogenate to ensure the activity is within the assay range. Alternatively, for limited or fixed sample amounts, incubation time in Step 3 can be increased.
  - For soil samples, we suggest testing several incubation lengths (1 - 24 hr) to ensure activity is within the assay range.
  - To relate urease activity to protein amount, measure protein concentration using BCA Protein Assay Kit II (Cat. # K813 or equivalent).
2. **Standard Curve Preparation:** Dilute Ammonium Chloride Standard to 1 mM by adding 10 µl of 100 mM Ammonium Chloride Standard to 990 µl of dH<sub>2</sub>O. Mix well. Add 0, 4, 8, 12, 16, and 20 µl of Standard into a series of wells in a 96-well plate to generate 0, 4, 8, 12, 16, and 20 nmol Standard per well. Adjust the volume to 100 µl/well with dH<sub>2</sub>O.
3. **Reaction Mix:** Prepare enough Reaction Mix for the number of wells (positive control, samples and reagent background control) to be analyzed. For each reaction, prepare 90 µl reaction mix:



	Reaction Mix
Urease Assay Buffer	88 $\mu$ l
Urea (1X)	2 $\mu$ l

Mix and add 90  $\mu$ l of the reaction mix into each well (except Standards and soil sample), mix well. Incubate plate at 37°C for 30 min.

- Measurement:** Add 80  $\mu$ l of Reagent 1 into each well and mix. Add 40  $\mu$ l of Reagent 2 into each well and mix. Incubate at 37°C for 30 min. Measure OD at 670 nm in a microplate reader as endpoint.
- Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Standard Curve. Subtract reagent background control reading from positive control and sample (enzyme, cell) readings. For soil samples\*, subtract soil background control reading from soil sample reading. Apply sample's corrected OD to the Standard Curve to get B nmol of ammonia generated by urease during the reaction time.

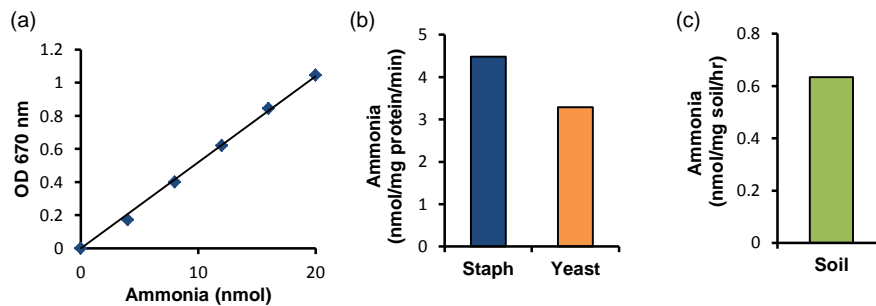
**Sample (cells and purified enzyme) Urease Activity (A) = B/(T x P) = nmol/mg protein/min = mU/mg protein**

Where: **B** is amount of ammonia generated in the sample well from Standard Curve (nmol)  
**P** is the protein concentration (mg)  
**T** is reaction time (min)

**\* Soil Sample Urease Activity (A) = B/(T x 5) = nmol/mg soil/hr**

Where: **B** is amount of ammonia generated in the sample well from Standard Curve (nmol)  
**5** is mg of soil measured in the assay  
**T** is reaction time (hr)

**Unit Definition:** One unit of Urease is the amount of enzyme that generates 1.0  $\mu$ mol of ammonia per min. at pH 7 at 37°C.



**Figure:** (a) Ammonia Standard curve (b) Measurement of Urease activity in *Staphylococcus aureus* and *Saccharomyces cerevisiae* (yeast) cell extracts. (c) Measurement of Urease activity in soil. Assays were performed according to the kit protocol.

#### VIII. RELATED PRODUCTS:

Urea Colorimetric Assay Kit (K375)	Ammonia Colorimetric Assay Kit (K370)
Urea Colorimetric Assay Kit II (K376)	Ammonia Colorimetric Assay Kit II (K470)
Uric Acid Colorimetric/Fluorometric Assay Kit (K608)	Phosphate-Buffered Saline (2113)
BCA Protein Assay Kit II (K813)	EZLys™ Bacterial Protein Extraction Reagent (8001)
Protease Inhibitor Cocktail (K271)	EZLys™ Yeast Protein Exaction Reagent (8003)
BCA Protein Assay Kit-Reducing agent Compatible (K818)	
BCA Protein Assay Kit (Test Tube)-Reducing agent Compatible (K819)	

**FOR RESEARCH USE ONLY! Not to be used on humans.**