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Product Information

Urease Activity Assay Kit

Catalog Number **MAK120**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Urease is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Urease is found in bacteria, yeast, and certain higher plant species. Urease activity is found in environmental and fecal samples.

The Urease Activity Assay kit provides a simple and direct procedure for measuring urease activity in biological and environmental samples. In this assay, urease catalyzes the hydrolysis of urea resulting in the production of ammonia. The ammonia is determined by the Berthelot method resulting in a colorimetric product measured at 670 nm, proportionate to the urease activity present in the sample. One unit of urease is the amount of enzyme that catalyzes the formation of 1.0 μmole of ammonia per minute at pH 7.0.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer, pH 7.0 Catalog Number MAK120A	20 mL
Urea Catalog Number MAK120B	1.5 mL
Ammonium Chloride, 50 mM Catalog Number MAK120C	100 μL
Reagent A Catalog Number MAK120D	12 mL
Reagent B Catalog Number MAK120E	6 mL

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- Clear 96-well flat-bottom plate
- 10 kDal Molecular Weight Cut-Off Filter (Catalog Number Z677108 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature. Storage at 2–8 °C, protected from light, is recommended.

Procedure

Sample Preparation

Soil and other samples can be extracted in Assay Buffer (10 mM sodium phosphate, pH 7.0) using any established methods.

Notes: The presence of ammonia in samples will result in assay background. Ammonia in samples can be removed by dialysis or filtration with a 10 kDa Molecular Weight Cut-Off Filter.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Sample Blank Preparation

Certain samples, such as soil samples, may contain low concentrations of ammonia. To correct for sample ammonia, immediately prior to assay step 4, prepare a sample blank by mixing the following reagents in the indicated order: 100 μL of Reagent A, 90 μL of sample extract, 10 μL of urea, and 50 μL of Reagent B. Continue with assay as written.

Ammonium Chloride Standards

Bring all components to room temperature before use. Dilute 5 µL of the 50 mM Ammonium Chloride Standard with 495 µL of Assay Buffer to create a 500 µM Ammonium Chloride Standard Solution. Dilute the 500 µM Ammonium Chloride Solution as indicated in Table 1 to create the standard curve.

Table 1.
Ammonium Standards

Number	NH_4Cl Standard (500 µM)	Buffer	Final Ammonia Concentration
1	100 µL	0 µL	500 µM
2	80 µL	20 µL	400 µM
3	60 µL	40 µL	300 µM
4	40 µL	60 µL	200 µM
5	30 µL	70 µL	150 µM
6	20 µL	80 µL	100 µM
7	10 µL	90 µL	50 µM
8	0 µL	100 µL	0 µM

Assay Reaction

1. Transfer 90 µL of the standards into separate wells of a 96 well plate.

2. Transfer 90 µL of samples into separate wells of the plate. Samples should be diluted in Assay Buffer. Transfer 90 µL of Assay Buffer into a separate well to serve as an Assay Blank.

3. Add 10 µL of Urea to each well. Incubate at room temperature for 10 minutes. For samples with low suspected urease activity, for example some environmental samples), incubate reaction for 2–4 hours at either 30 °C or 37 °C.
   Note: Certain samples, such as soil samples, may contain low concentrations of ammonia. To correct for sample ammonia, prepare a Sample blank by mixing the following reagents in the indicated order: 100 µL of Reagent A, 90 µL of sample extract, 10 µL of urea, and 50 µL of Reagent B.

4. Add 100 µL of Reagent A to each well to terminate the Urease reaction, tap plate to mix, and then add 50 µL of Reagent B to each well. Tap plate to mix again.

5. Incubate the reaction for 30 minutes protected from light.

6. Measure the absorbance at 670 nm (A_{670}).

These assays can be adapted for use in standard 1 mL cuvettes. To adapt to cuvettes follow protocol as for 96 well plates but scale up volumes as follows: 360 µL of sample, 40 µL of urea, 400 µL of Reagent A, and 200 µL of Reagent B.

Results

Calculations

Use the values obtained from the appropriate ammonium chloride standards to plot a standard curve and determine the slope using linear regression fitting.

Note: A new standard curve must be set up each time the assay is run.

The amount of urease enzymatic activity in the sample can be calculated using the equation below.

$$\text{Urease Activity (units/L)} = \frac{(A_{670})_{sample} - (A_{670})_{blank}}{\text{Slope} \times t} \times n$$

where:

n = dilution factor

t = incubation time (10 minutes for standard assay).

$(A_{670})_{blank}$ = value for Assay Blank (Assay Buffer alone)

Note: If Urease activity is higher than 25 units/L, dilute sample in assay buffer, repeat assay, and multiply by dilution factor.

One unit of urease is the amount of enzyme that catalyzes the formation of 1.0 μmole of ammonia per minute at pH 7.0.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold reagents	Bring components to room temperature prior to starting assay
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in incompatible buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare reagents fresh for each assay
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further

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