

Urease (UE) Activity Assay Kit (IPB, Micro Method)

Cat. No.: U1521990 | Pack size: 48 T; 96 T | Storage: 2-8°C; protected from light

Overview

Urease (UE, EC 3.5.1.5) catalyzes the hydrolysis of urea to produce ammonia and carbonic acid. UE activity is positively correlated with organic matter content, total nitrogen and available nitrogen levels, serving as an indicator of soil and tissue nitrogen status.

This kit adopts indophenol blue colorimetry to quantify $\text{NH}_3\text{-N}$ generated from urea hydrolysis by urease, with maximum absorbance at 630 nm.

Contents & Storage

Item No.	Appearance	Components	48 T	96 T	Storage
U1521990A	Liquid	Extraction Solution	70 mL	70 mL×2	2-8°C
U1521990B	Powder	Reagent I	1 vial	1 vial	2-8°C
U1521990C	Liquid	Reagent II	25 mL	50 mL	2-8°C
U1521990D	Liquid	Reagent IIIA	0.4 mL	0.8 mL	2-8°C
U1521990E	Liquid	Reagent IIIB	1.6 mL	3.2 mL	2-8°C
U1521990F	Liquid	Reagent IV	2 mL	4 mL	2-8°C. Store in the dark.
U1521990G	Liquid	Standard	0.2 mL	0.4 mL	2-8°C

Notes

1. Verify the volume of each kit component before starting experiments.
2. All reagents are supplied with an extra 10% volume on top of the standard specification for standard curve preparation and preliminary trial tests.

Self-Supplied Instruments and Reagents

Microplate reader, transparent 96-well microplates, PBS, deionized water, tissue homogenizer (for tissue samples), constant temperature incubator, refrigerated centrifuge, adjustable pipettes and pipette tips.

General Precautions

1. Conduct preliminary trials with 2-3 samples exhibiting expected large differences before formal detection.

2. For tissue, cell and bacterial samples, normalize final results by total protein concentration to eliminate inter-sample variation.
3. This kit is compatible with spectrophotometer detection; proportionally adjust reagent volumes following spectrophotometer operating requirements.
4. It is recommended to generate an independent standard curve for each experiment to improve detection accuracy. If no self-made standard curve is available, calculate results using the typical standard curve formula provided in the Results section.
5. Biochemical assay reagents are generally irritant and biohazardous. Wear full personal protective equipment including lab coat, mask, gloves and hairnet throughout all operations, and perform all experimental steps in a fume hood or biosafety cabinet for occupational safety.
6. This product is for research use only; not intended for clinical diagnostic applications.

Experimental Procedures

1. Reagent Preparation

Reagent Name	Preparation Instructions	Notes & Precautions
Extraction Solution	Ready-to-use; equilibrate to room temperature before use	Store at 2-8°C
Working Reagent I	Prepare immediately before use; for 48 T, add 9 mL deionized water; for 96 T, add 18 mL deionized water, fully dissolve before use	Store at 2-8°C
Reagent II	Ready-to-use; equilibrate to room temperature before use	Store at 2-8°C
Working Reagent III	Prepare immediately before use; pour Reagent III A into Reagent III B and mix well before use	Stable for 1 week when stored at 4°C; toxic and with pungent odor, perform all operations in a fume hood
Reagent IV	Ready-to-use; equilibrate to room temperature before use	Store at 4°C, protected from light
Standard	1 M standard solution, ready-to-use; equilibrate to room temperature before use	Store at 2-8°C

2. Standard Working Solution Preparation

Dilute 1 M Standard with Extraction Solution to prepare a 1 mM stock standard. Further serially dilute the 1 mM standard solution as specified in the table below. Prepare and analyze a standard curve alongside every independent experiment. Diluted standard solutions are unstable and must be used within 4 hours of preparation.

No.	Standard (μL)	Extraction Solution (μL)	Concentration (μM)
1	200 μL of 1 mM standard	0	1000
2	100 μL of 1000 μM standard	100	500
3	100 μL of 500 μM standard	100	250
4	100 μL of 250 μM standard	100	125
5	100 μL of 125 μM standard	100	62.5
6	100 μL of 62.5 μM standard	100	31.25
7	100 μL of 31.25 μM standard	100	15.625
Blank	0	100	0

3. Sample Preparation

Notes: Fresh samples are recommended for immediate detection. If testing cannot be performed right away, samples may be stored at -80°C for up to 1 month.

Animal & Plant Tissues: Weigh approximately 0.1 g tissue sample, add 1 mL Extraction Solution, homogenize on ice, centrifuge at 8000 g, 4°C for 10 min. Collect the supernatant and keep on ice prior to assay.

Cells or Bacteria: Harvest 5×10^6 cells/bacteria, wash with cold PBS, centrifuge at 800 g for 2 min and discard supernatant. Resuspend pellet in 1 mL Extraction Solution, lyse by sonication on ice for 5 min (20% power / 200 W, 3 s sonication / 7 s interval, repeat 30 cycles). Centrifuge lysate at 8000 g, 4°C for 10 min, collect supernatant and store on ice.

Serum / Plasma: Detect directly without pre-processing.

4. Assay Protocol

(1) Microplate reader setup: Preheat the instrument for ≥ 30 min and set detection wavelength to 630 nm.

(2) Enzymatic reaction: Prepare reaction mixtures in 1.5 mL EP tubes as outlined below:

Reagent	Assay Tube (μL)	Control Tube (μL)
Sample	20	20
Working Reagent I	90	0
Deionized Water	0	90
Reagent II	190	190

(3) Thoroughly mix all tubes, incubate in a 37°C water bath for 1 h, then centrifuge at 10000 g, 25°C for 10 min. Collect supernatant and dilute 10-fold (mix 0.1 mL supernatant with 0.9 mL deionized water).

(4) Ammonia quantification: Pipette reagents into a 96-well plate following the plate layout below (blank and standard wells only require single measurement per run).

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)	Blank Well (μL)
Diluted Supernatant	80	80	0	0
Standard Solution	0	0	80	0
Deionized Water	0	0	0	80
Working Reagent III	15	15	15	15
Reagent IV	15	15	15	15
Deionized Water	90	90	90	90

Notes: Mix thoroughly after adding Reagent IV, then incubate at room temperature for 20 min.

(5) Absorbance measurement: Mix each well rapidly, read absorbance at 630 nm, record values as A_{sample} , A_{control} , A_{standard} and A_{blank} respectively.

Calculation of Urease Activity

Two equivalent formulas are provided below: the full derivation formula and simplified computational formula.

1. Raw Data Calculation

$$\Delta A_{\text{sample}} = A_{\text{sample}} - A_{\text{control}}$$

$$\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$$

2. Standard Curve Construction

Plot standard curve with standard concentration as the y-axis and $\Delta A_{\text{standard}}$ as the x-axis. Substitute ΔA_{sample} into the curve equation to obtain y value (unit: μM).

3. Urease Activity Calculation

Calculation by Tissue Weight

Unit definition: One unit (U) refers to the amount of urease that produces 1 μmol NH₃-N per gram of tissue per minute.

$$\text{UE (U/g)} = y \times (V_{\text{total-reaction}} \div V_{\text{sample}}) \times 10 \times V_{\text{extraction}} \div T \div 1000 \div W = 0.0025y \div W$$

Calculation by Total Protein Concentration

Unit definition: One unit (U) refers to the amount of urease that produces 1 μmol NH₃-N per milligram of total protein per minute.

$$UE \text{ (U/mg prot)} = y \times (V_{\text{total-reaction}} \div V_{\text{sample}}) \times 10 \div T \div 1000 \div \text{Cpr} = 0.0025y \div \text{Cpr}$$

Calculation by Cell/Bacterial Count

Unit definition: One unit (U) refers to the amount of urease that produces 1 μmol $\text{NH}_3\text{-N}$ per 10^6 cells or bacteria per minute.

$$UE \text{ (U/10}^6\text{)} = y \times (V_{\text{total-reaction}} \div V_{\text{sample}}) \times 10 \times V_{\text{extraction}} \div T \div 1000 \div N = 0.0025y \div N$$

Calculation by Serum/Plasma Volume

Unit definition: One unit (U) refers to the amount of urease that produces 1 μmol $\text{NH}_3\text{-N}$ per milliliter of serum/plasma per minute. T: Enzymatic reaction incubation time = 60 min.

$$UE \text{ (U/mL)} = y \times (V_{\text{total-reaction}} \div V_{\text{sample}}) \times 10 \div T \div 1000 = 0.0025y$$

V_{total-reaction}: Total volume of enzymatic reaction system = 0.3 mL.

V_{sample}: Volume of sample added to reaction = 0.02 mL.

1000: Unit conversion factor (1 L = 1000 mL).

Cpr: Total protein concentration of sample (mg/mL).

10: 10-fold dilution factor for post-reaction supernatant.

V_{extraction}: Volume of Extraction Solution used for sample lysis = 1 mL.

W: Weight of tissue sample (g).

N: Cell/bacterial count in units of 10^6 (e.g., 5×10^6 cells corresponds to $N = 5$).

Representative Results

Typical Standard Curve

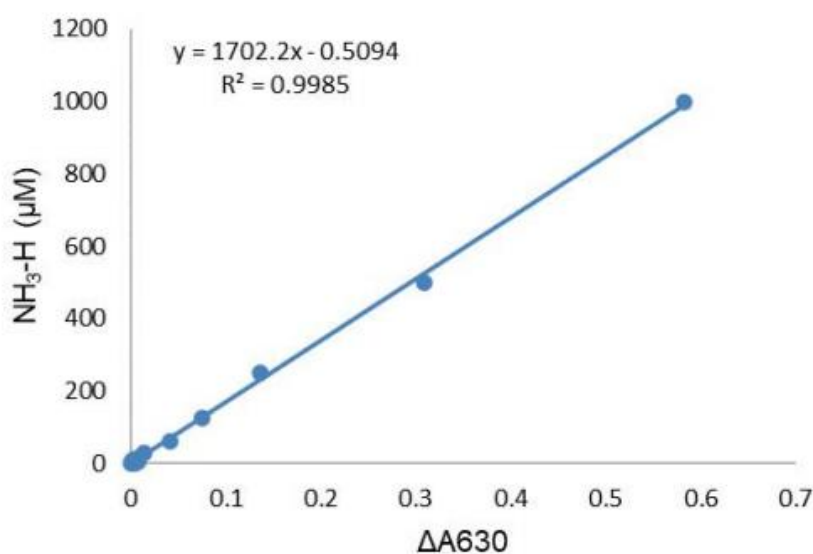


Figure 1. Typical standard curve for the Urease Activity Assay Kit.

Calculation Examples

1. Tissue sample example: 0.1 g mouse liver tissue was assayed in 96-well plate.

$\Delta A_{\text{sample}} = 0.206 - 0.09 = 0.116$. Substitute into standard curve to get $y = 196.995$. $UE \text{ (U/g)} = 0.0025 \times 196.995 \div 0.1 = 4.924 \text{ U/g}$.

2. Cell sample example: 1×10^6 Jurkat cells were assayed.

$\Delta A_{\text{sample}} = 0.110 - 0.044 = 0.066$. Substitute into standard curve to get $y = 111.7753$, $N = 5$. $UE \text{ (U/} 10^6 \text{ cells)} = 0.0025 \times 111.7753 \div 5 = 0.056 \text{ U/} 10^6$.

3. Serum example: 20 μL bovine serum was assayed.

$\Delta A_{\text{sample}} = 0.210 - 0.093 = 0.117$. Substitute into standard curve to get $y = 198.689$. $UE \text{ (U/mL serum)} = 0.0025 \times 198.689 = 0.497 \text{ U/mL}$.

FAQ

What to do if measured ΔA_{sample} is excessively high or low?

If $\Delta A_{\text{sample}} > 0.6$: Dilute the original sample with Extraction Solution or reduce the starting sample weight/cell number, then re-assay.

If $\Delta A_{\text{sample}} < 0.001$: Increase the mass/volume of starting sample appropriately before re-testing.

Storage and Shipping

Item	Information
Storage	Store at 2-8°C, Protected from light
Shipped In	Wet ice
Stability And Storage	Store at 2-8°C long term (12 months). Store in the dark.

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