EZMeta™ Urease Colorimetric Activity Kit

Cat #: D-AKC3070

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Animal and Plant Tissue, Cell, Bacteria, Serum, Plasma

Assay Principle

Urease (UE) can hydrolyze urea to produce ammonia and carbonic acid. UE activity was positively correlated with organic matter content, total nitrogen and available nitrogen content, reflecting the nitrogen status. EZMeta™ Urease Colorimetric Activity Kit provides a simple, convenient and rapid UE activity detection method, which is suitable for the detection of animal and plant tissue, cell, bacteria, serum, plasma and other samples. The detection principle is that the NH_3-N produced by urease hydrolysis of urea is determined by indophenol blue colorimetry, and there is a characteristic absorption peak at 630 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Solution	60 mL	120 mL	4°C
Reagent Ⅰ	1	1	4°C
Reagent Ⅱ	22 mL	44 mL	4°C
Reagent ⅢA	0.4 mL	0.8 mL	4°C
Reagent ⅢB	1.6 mL	3.2 mL	4°C
Reagent Ⅳ	2 mL	4 mL	4°C, protected from light
NH_4Cl Standard （1 M）	0.2 mL	0.4 mL	4°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 630 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Ice maker, centrifuge, water bath

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅰ: Before use, add 9 mL deionized water for 48T; add 18 mL deionized water for 96T; Fully dissolve it. Store at 4°C.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

ReagentⅢ: Before use, pour liquid A into liquid B and mix it for use, and store the inexhaustible reagents at 4°C for a week.

ReagentⅣ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Standard Curve Setting: 1 M NH_4Cl Standard was diluted to 1 mM with Extraction Solution. Dilute the 1 mM NH_4Cl

Standard with Extraction Solution to 500, 250, 125, 62.5, 31.25, 15.625 μM as indicated in the table below.

Num.	Volume of Standard	Volume of Extraction Solution(μL)	The Concentration of Standard (μM)
Std.1	200 μL of Standard	0	1,000
Std.2	100 μL of Std.1 (1,000 μM)	100	500
Std.3	100 μL of Std.2 (500 μM)	100	250
Std.4	100 μL of Std.3 (250 μM)	100	125
Std.5	100 μL of Std.4 (125 μM)	100	62.5
Std.6	100 μL of Std.5 (62.5 μM)	100	31.25
Std.7	100 μL of Std.6 (31.25 μM)	100	15.625

Sample Preparation

Note: Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80°C for 1 month.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Solution and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Cell (bacteria): Collect 5×10^6 cell or bacteria into the centrifuge tube, wash with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Solution to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Serum (plasma): Direct detection.

Note: It will be better to quantify the total protein with EZMeta™ Urease Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 630 nm, visible spectrophotometer was returned to zero with deionized water.

2. Enzymatic reaction (the following operations are performed in a 1.5 mL centrifuge tube).

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

Mix well, put it in a water bath at 37°C for 1 h, centrifuge at 10,000 g at 25°C for 10 min, and take the supernatant.

3. Dilute the supernatant 10 times (take 0.1 mL supernatant and add 0.9 mL deionized water).

4. Determination of ammonia (the following operations are performed in 96-well plates or microglass cuvette):

Reagent	Test Well (μL)	Control Well (μL)	Standard Well(μL)	Blank Well(μL)
Diluted Supernatant	80	80	0	0
Standard	0	0	80	0
Deionized Water	0	0	0	80
ReagentⅢ	15	15	15	15
ReagentⅣ	15	15	15	15
Mix well and place 20 min at room temperature.				
Deionized Water	90	90	90	90

After rapid mixing, the absorbance value was determined by 630 nm, which was recorded as A_{Test}, $A_{Control}$, $A_{Standard}$, A_{Blank}, calculated $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, the sample can be appropriately diluted with Extraction Solution, the calculated

result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the Standard Solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μM).

2. Calculation of UE activity of samples

(1) Calculation of UE activity in serum (plasma):

Definition of unit: each mL serum (plasma) produces 1 μmol of NH_3-N per min is defined as an enzyme activity unit.

UE (U/mL)=y×($V_{Total\ reaction}$÷V_{Sample})×10÷T÷1,000=**0.0025y**

(2) Calculation of UE activity in tissues

a. Calculated according to the concentration of sample protein

Definition of unit: 1 μmol of NH_3-N produced per mg tissue protein per min is defined as an enzyme activity unit.

UE (U/mg prot)=y×($V_{Total\ reaction}$÷V_{Sample})×10÷T÷1,000÷Cpr=**0.0025y÷Cpr**

b. Calculated according to sample fresh weight

Definition of unit: 1 μmol of NH_3-N produced per g of tissue per min is defined as an enzyme activity unit.

UE (U/g fresh weight)=y×($V_{Total\ reaction}$÷V_{Sample})×10×$V_{Total\ sample}$÷T÷1,000÷W=**0.0025y÷W**

c. Calculated according to cell (bacterial) density

Definition of unit: every 1 million cells (bacteria) produces 1 μmol of NH_3-N per min is defined as an enzyme activity unit.

UE ($U/10^6$)=y×($V_{Total\ reaction}$÷V_{Sample})×10×$V_{Total\ sample}$÷T÷1,000÷n=**0.0025y÷n**

Where: $V_{Total\ reaction}$: Total volume of reaction system, 0.3 mL; V_{Sample}: Add sample volume, 0.02 mL; T: Reaction time, 60 min; 1,000: Unit conversion coefficient, 1L=1,000 mL; Cpr: Protein concentration, mg/mL; $V_{Total\ sample}$: Extract solution volume, 1 mL; W: Sample quality, g; n: Total cell (bacteria), million.

Typical Data

Typical standard curve:

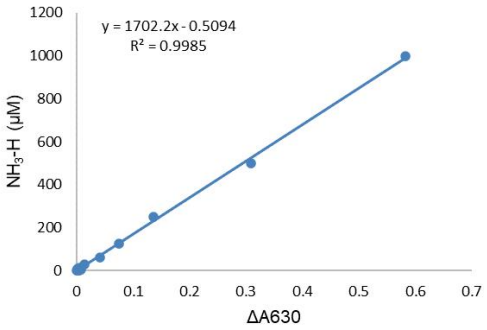

Figure1. Standard Curve for NH_3-N.

Examples:

1. Take 0.1 g mouse liver and use 96-well plate to calculate ΔA_{Test}=0.206-0.09=0.116, y=196.985. The content calculated according to the sample quality is as follows:

UE (U/g fresh weight)=0.0025×196.985÷0.1=4.925 U/g.

2. Take 5 million Jurkat cells and use 96-well plate to calculate ΔA_{Test} =0.110-0.044=0.066, y=111.753. The content calculated according to the cell density is as follows:

UE (U/10^6)=0.0025×111.753÷5=0.056 U/10^6.

3. Take 20 μL bovine serum and use 96-well plate to calculate ΔA_{Test}=0.210-0.093=0.117, y=198.689. The content calculated according to the liquid volume is as follows:

UE (U/mL)=0.0025×198.689÷5=0.497 U/mL.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.