

Urease (UE) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0347

Size:100T/48S

Components:

Extract solution: 60ml×1 bottle, storage at 4°C .

Reagent I: powder×1 bottle, storage at 4°C . dissolve with 3ml of distilled water before use. **Reagent II:** 10ml×1 bottle, storage at 4°C and protected from light.

Reagent III A: 0.4 ml×1 bottle, storage at 4°C .

Reagent III B: 1.6 ml×1 bottle, storage at 4°C . Add Reagent 3A to Reagent 3B, mix for use (name reagent III),

Reagent IV: 2 ml×1 bottle, storage at 4°C .

Standard: 1ml×1 bottle, storage at 4°C . 1mg/ml nitrogen standard solution.

Product Description:

Urease (UE) is widely distributed in the seeds of plants, also in the blood and urine of animals. Some microorganisms can also secrete urease. UE can hydrolyze urea to ammonia and carbonic acid, which plays a key role in urea transformation. The UE activity can be determined by calculating the content of NH₃-N with indophenol blue colorimetry.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, micro glass cuvette/ 96 well flat-bottom plate, constant temperature water bath, low temperature centrifuge, balance, mortar/homogenizer.

Sample preparation:

- I. Bacteria or cells: Number of cells / bacteria (10^4): volume of extract solution (mL) is 500-1000:1. Suggested 5 million with 1mL of extract solution. Splitting bacteria or cells with ultrasonication (ice bath, power 300W, work time 3s , interval 7s , repeat 3 min), centrifuge at 12000g and 4C for 15min, supernatant on ice is used for test.
- II. Tissue: Mass (g): extraction volume (mL) is 1:5- 10. Add 1 ml of extract solution into 0. 1g of tissue, fully grinding on ice. centrifuge at 12000g and 4C for 15min, supernatant on ice is used for test
- III. Serum/ plasma: Detect directly.

Procedure:

1. Preheat Spectrophotometer/ microplate reader for 30min, adjust the wavelength to 630 nm, set the counter to zero with distilled water.
2. Dilute 1mg/mL nitrogen standard solution to 2ug/mL with distilled water for use.

3. Add the following reagents:

Reagent name(ul)	Blank tube (A2)	Standard tube (A1)	Test tube (A3)	Contrast tube (A4)
Sample	-	-	20	20
Distilled water	-	-		40
Reagent I	-	-	40	-
Reagent II	-	-	80	80
Mix thoroughly form mixture, react at 37C for 1 hour, add the following in EP tube or 96 well plate.				
Mixture	-	-	80	80
Distilled water	80	-		
Standard	-	80		
Reagent III	16	16	16	16
Reagent IV	12	12	12	12
Mix thoroughly, stand at RT for 20min.				
Distilled water	92	92	92	92
Mix thoroughly, detect absorbance at 630nm, $\Delta A(\text{standard})=\Delta A(S)=A1-A2$, $\Delta A(\text{test})=\Delta A(T) =A3-A4$.				

Calculation:

1. Liquid:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1ug of NH₃-N in per min every ml liquid.

$$UE(U/mL)= \Delta A(T) \div \Delta A(S) \times C \times V \div V_S \div T = 0.233 \times \Delta A(T) \div \Delta A(S)$$

2. Tissue, bacteria or cell:

a. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1ug of NH₃-N in per min every mg tissue protein.

$$UE (U/mg \text{ prot})= \Delta A(T) \div \Delta A(S) \times C \times V \div (C_{pr} \times V_S) \div T = 0.233 \times \Delta A(T) \div \Delta A(S) \div C_{pr}$$

b. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1ug of NH₃-N in per min every gram tissue.

$$UE(U/g)= \Delta A(T) \div \Delta A(S) \times C \times V \div (W \times V_S \div V_e) \div T = 0.233 \times \Delta A(T) \div \Delta A(S) \div W$$

c. Density of bacteria or cell:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1ug of NH₃-N in per min every 1 million bacteria or cells.

$$UE (U/10^6 \text{ cell})= \Delta A(T) \div \Delta A(S) \times C \times V \div (N \div V_S \times V_e) \div T = 0.233 \times \Delta A(T) \div \Delta A(S) \div N$$

C: standard concentration, 2ug/ mL

Cpr: Sample concentration (mg/mL);

W: Sample weight(g);

Vs: Sample volume (mL), 0.02 mL;
V: Enzyme reaction volume, 0.14 mL;
Ve: Extraction volume, 1 mL;
T: Reaction time (min), 60 min.
N: cell or bacteria amount, 1 million.

Note:

Dilute the mixture or sample with distilled water before detecting if the $\Delta A > 0.6$.

Experimental Examples:

- 1、 Take 0.1g of *Vigna radiata* and add 1mL extract for sample processing, take the supernatant and operate according to the measurement procedure, measure by the 96 well plate and calculate $\Delta A = A_3 - A_4 = 0.152 - 0.092 = 0.047$, $\Delta A_s = A_1 - A_2 = 0.241 - 0.041 = 0.200$, calculate the enzyme based on the sample weight:
UE Activity (U/g weight) = $0.233 \times \Delta A \div \Delta A_s \div W = 0.233 \times 0.047 \div 0.2 \div 0.1 = 0.548$ U/g weight.
- 2、 Take 0.1g of kidney and add 1mL extract for sample processing, take the supernatant and operate according to the measurement procedure, measure by the 96 well plate and calculate $\Delta A = A_3 - A_4 = 0.215 - 0.162 = 0.053$, $\Delta A_s = A_1 - A_2 = 0.241 - 0.041 = 0.200$, calculate the enzyme based on the sample weight :
UE Activity (U/g weight) = $0.233 \times \Delta A \div \Delta A_s \div W = 0.233 \times 0.053 \div 0.2 \div 0.1 = 0.6175$ U/g weight.
- 3、 Take 20 μ L of turkey serum and directly follow the measurement procedure, and calculate $\Delta A = 0.145 - 0.097 = 0.048$, $\Delta A_s = A_1 - A_2 = 0.241 - 0.041 = 0.200$, calculate the enzyme based on the sample volume
UE Activity (U/mL) = $0.233 \times \Delta A \div \Delta A_s = 0.233 \times 0.048 \div 0.2 = 0.0559$ U/mL.

Related Products:

AK0301/AK0300 Nitrate Reductase (NR) Activity Assay Kit
AK0436/AK0435 Glutaminase (GLS) Activity Assay Kit
AK0434/AK0433 Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit