Urease (UE) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: BC4115

Size:100T/48S

Components:

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

Reagent I: powder×1 bottle, storage at 4℃. dissolve with 3ml of distilled water before use.

Reagent II: 10ml×1 bottle, storage at 4℃ and protected from light.

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

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

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

Standard: 1ml×1 bottle, storage at 4℃. 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 4℃ 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 4℃ 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 37℃ 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, ΔA(standard)=ΔA(S)=A1-A2, ΔA(test)=ΔA(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 NH_3-N in per min every ml liquid.

   UE(U/mL)= ΔA(T) ÷ΔA(S) ×C×V÷ Vs÷T=0.233 ×ΔA(T) ÷ΔA(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 NH_3-N in per min every mg tissue protein.

   UE (U/mg prot)= ΔA(T) ÷ΔA(S) ×C×V÷ (Cpr×Vs)÷T=0.233×ΔA(T) ÷ΔA(S) ÷Cpr

   b. Sample weight:

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1ug of NH_3-N in per min every gram tissue.

   UE(U/g)= ΔA(T) ÷ΔA(S) ×C×V÷(W× Vs÷Ve) ÷T=0.233×ΔA(T) ÷ΔA(S) ÷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 NH_3-N in per min every 1 million bacteria or cells.

   UE (U/10^6 cell)= ΔA(T) ÷ΔA(S) ×C×V÷(N÷Vs×Ve)÷T=0.233×ΔA(T) ÷ΔA(S) ÷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 ΔA>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 ΔA=A3-A4=0.152-0.092=0.047，ΔAs=A1-A2=0.241-0.041=0.200，calculate the enzyme based on the sample weight:

UE Activity（U/g weight）=0.233×ΔA÷ΔAs÷W=0.233×0.047÷0.2÷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 ΔA=A3-A4=0.215-0.162=0.053，Δs=A1-A2=0.241-0.041=0.200，calculate the enzyme based on the sample weight：

UE Activity（U/g weight）=0.233×ΔA ÷ΔAs÷W=0.233×0.053÷0.2÷0.1=0.6175 U/g weight。

3、 Take 20μL of turkey serum and directly follow the measurement procedure, and calculate ΔA =0.145-0.097=0.048，ΔAs=A1-A2=0.241-0.041=0.200，calculate the enzyme based on the sample volume

UE Activity（U/mL=0.233×ΔA÷ΔAs=0.233×0.048÷0.2=0.0559 U/mL。

Related Products:

BC0080/BC0085 Nitrate Reductase (NR) Activity Assay Kit

BC1450/BC1455 Glutaminase (GLS) Activity Assay Kit

BC1460/BC1465 Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit