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Urease (UE) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0348

Size:50T/24S

Components:

Extract solution: 30ml×1 bottle, storage at 4℃ .

Reagent I: powder×1 bottle, storage at 4℃ . dissolve with 6ml of distilled water before use.

Reagent II: 25ml×1 bottle, storage at 4℃and protected from light.

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

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

Reagent IV: 5ml×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, balance, water bath, low temperature centrifuge, 1ml glass cuvette, mortar.

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 cell with ultrasonication (ice bath, power 300W, work time 3s， interval 7s， repeat for 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 and fully grind 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 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:

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Reagent name(ul)	Blank tube (A2)	Standard tube (A1)	Test tube (A3)	Contrast tube (A4)
Sample	-	-	100	100
Distilled water	-	-	-	200
Reagent I	-	-	200	-
Reagent II	-	-	400	400
Mix thoroughly form mixture, react at 37℃ for 1 hour.				
Mixture	-	-	400	400
Distilled water	400	-	-	-
Standard	-	400	-	-
Reagent III	80	80	80	80
Reagent IV	60	60	60	60
Mix thoroughly, stand at RT for 20min.				
Distilled water	460	460	460	460
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:**

   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

   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

   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), 0.1 mL;
V: Enzyme reaction volume, 0.7 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>1.

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, and calculate ΔA=A3-A4=0.221-0. 102=0. 119 , ΔAs=A1-A2=0.342-0.005=0.337， calculate the enzyme based on the sample weight:

UE Activity (U/g weight) =0.233×ΔA÷ΔAs÷W=0.233×0. 119÷0.337÷0. 1=0.8228 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, and calculate ΔA=A3-A4=0.315-0.226=0.089， Δs=A1-A2=0.342-0.005=0.337， calculate the enzyme based on the sample weight：

UE Activity (U/g weight) =0.233×ΔA ÷ΔAs÷W=0.233×0.089÷0.337÷0. 1=0.6153 U/g weight。

3、 Take 100μL of turkey serum and directly follow the measurement procedure, and calculate ΔA =A3-A4=0.210-0. 125=0.085， ΔAs=A1-A2=0.342-0.005=0.337， calculate the enzyme based on the sample volume

UE Activity (U/mL) =0.233×ΔA ÷ΔAs=0.233×0.085÷0.337=0.0588 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