Uricase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0285

Size:100T/48S

Components:

Extract Solution: Liquid 60 mL×1, storage at 4℃.

Reagent I: Liquid 30 mL×1, storage at 4℃.

Reagent II: Powder×2, storage at 4℃ and protect from light. Add 2 mL of Reagent I when the solution will be used. The unused reagents can be stored at 4℃ for one week.

Reagent III: Powder×1, storage at 4℃ and protect from light. Add 6 mL of Reagent I when the solution will be used. The unused reagents can be stored at 4℃ for two week.

Reagent IV: Powder×1, storage at 4℃ and protect from light. Add 4 mL of Reagent I when the solution will be used. The unused reagents can be stored at 4℃ for one week.

Reagent V: Powder×1, storage at -20℃ and protect from light. Add 6 mL of Reagent I when the solution will be used. The unused reagents can be stored at -20℃ for one week.

Reagent VI: Liquid 6 mL×1, storage at -20℃ and protect from light.

Standard: Liquid 102 μL×1, storage at 4℃ and protect from light. Add 898 μL of distilled water to 1 mmol/mL hydrogen peroxide standard solution when the solution will be used.

Preparation of working solution A: The solution is use for determination of samples Test tube, Contrast tube, and Standard tube.Reagent II, Reagent III, Reagent IV, Reagent V and Reagent VI are mixed in a 1: 1:1:1:2 ratio, prepared according to sample size. It is recommended to use up within 2 hours after mixing.

Preparation of working solution B: The solution is use for determination of samples Test tube, Contrast tube, and Standard tube.Reagent II, Reagent III, Reagent IV, Reagent V and Reagent I are mixed in a 1: 1:1:1:2 ratio, prepared according to sample size. It is recommended to use up within 2 hours after mixing.

Description:

Uricase, also known as uric acid oxidase, is an oxidase that participates in the purine degradation pathway. It can break down uric acid into allantoin and excrete it. Uric acid is the end product of purine metabolism. Excessive accumulation will lead to a variety of diseases such as ventilation, kidney disease and cardiovascular disease. Uricase is of great significance in the clinical detection and treatment of uric acid-related diseases.

Uricase catalyzes the decomposition of uric acid into allantoin, CO_2 and H_2O_2. H_2O_2 oxidizes Fe^{2+} in potassium ferrocyanide to form Fe^{3+}. Fe^{3+} further reacts with 4-aminoantipyrine and phenol to form red quinones, which has a characteristic absorption peak at 505 nm and reflects the activity of urase by measuring the absorbance at 505 nm.

Protocol:

I. Sample extraction:

Tissue:

Accordance the ratio of tissue(g) : extract solution volume (mL)=1: 5~10 (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Bacteria or cells:

Accordance the ratio of cells amount(10^4) : extract solution volume (mL)=500~1000: 1 (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 200w, ultrosonic 3s, interval 7s for 5 minutes). Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

II. Determination procedure

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 505 nm, set zero with distilled water.

2. Dilute 1 mmol/mL hydrogen peroxide standard solution with distilled water to 0.5 mmol/mL standard solution.

3. Add reagents with the following list:

Reagent (μL)	Contrast tube (C)	Test tube (T)	Standard tube (St)	Blank tube (B)
Sample	30	30	-	-
Standard solution	-	-	30	-
Distilled water	-	-	-	30
Working solution A	-	170	170	170
Working solution B	170	-	-	-
Mix well, react in water bath at 37℃(mammal) or 25℃ (other species) for 30 minutes. Determine the absorption value at 505 nm, record as A_C, A_T, A_{St} and A_B. Calculate $\Delta A=(A_T - A_C)$, $\Delta A_{St}=A_{St}-A_B$. Note: A control tube is required for each test tube. Testing of the same batch of samples, the soilless tube only need to be measured once or twice.				

III. Calculation of uricase activity:

1. Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzyme activity that per gram of tissue catalyze the hydrolyze of uric acid to produce 1 μmol of H_2O_2 per hour at pH8.8.

Uricase activity(U/g fresh weight)= $\Delta A \div (\Delta A_{St} \div C_{St}) \times V_S \div (W \times V_S \div V_E) \div T = \Delta A \div \Delta A_{St} \div W$

2. Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the hydrolyze of uric acid to produce 1 μmol of H_2O_2 per hour at pH8.8.

Uricase activity(U/g fresh weight)= $\Delta A \div (\Delta A_{St} \div C_{St}) \times V_S \div (Cpr \times V_S) \div T = \Delta A \div \Delta A_{St} \div Cpr$

3. Calculation according to cells or bacteria

Definition of unit: One unit is defined as an enzyme activity that per 1 0000 cells or bacteria catalyze the hydrolyze of 1 mg of starch per minute at pH8.8.

Uricase activity(U/g fresh weight)= $\Delta A \div (\Delta A_{St} \div C_{St}) \times V_S \div (N \times V_S) \div T = \Delta A \div \Delta A_{St} \div N$

C_{St} : Concentration of standard solution, 0.5 μmol/mL;
Vs: Sample volume, 0.03 mL;
V_E: Extract volume, 1 mL;
Cpr: Sample protein concentration (mg/mL);
N: The number of cells or bacteria, 10 thousand for one unit
T: Reaction time , 0.5 hour.

Note:

1. If A>1.5, please dilute the sample to appropriate concentration, multiply dilute times in the formular.
2. Working solution A and working solution B should be prepared according to the sample size, and it is recommended to use up within 2 hours. The working solution is pale yellow, and will change from pale yellow to pink, red, or even wine red over time. If discolored, it is considered invalid and needs reconfiguration.

Experimental Examples:

1. Take 0.1g of mouse liver, process the sample, take the supernatant and diluted 4 times, carry out the determination according to the operation steps. The calculation is: ΔA=At-Ac=0.804-0.285=0.519，ΔAst=Ast-Ab=0.741-0.051=0.690, calculate the enzyme activity according to sample weight:

Uricase Activity（U/g weight）= ΔA÷ΔAst÷W×4（diluted times）= 0.519÷0.690÷0.1×4=30.09 U/g weight

Related Products:

NA0359/NA0358 Tannase Activity Assay Kit
NA0357/NA0356 Cinnamic acid 4-hydroxylase(C4H) Activity Assay Kit
NA0351/NA0350 Anthocyanidin Reductase Activity Assay Kit
NA0349/NA0348 Indoleacetic acid oxidase Activity Assay Kit
NA0304/NA0303 Hephaestin(HP) Activity Assay Kit