Xanthine Oxidase(XOD) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC1095

Size:100T/96S

Components:

Reagent I: Liquid 120 mL×1. Store at 2-8℃.

Reagent II: Liquid 10 mL×1. Store at 2-8℃.

Reagent III: Powder×1. Store at 2-8℃. Add 2 mL of Reagent II into Reagent III and mix it well. Dilute it with Reagent II 5 times according to sample number before use. It could be stored at 2-8℃ for one week.

Reagent IV: Liquid 5 mL×1. Store at 2-8℃.

Reagent V: Liquid 8 mL×1. Store at 2-8℃.

Reagent VI: Liquid 8 mL×1. Store at 2-8℃.

Standard: Liquid 0.5 mL×1. Store at 2-8℃. 10 μmol/mL $NaNO_2$ standard solution.

Product Description:

XOD (EC 1.17.3.2) catalyzes the oxidation of hypoxanthine to xanthine and superoxide anion, which is one of the main sources of active oxygen and is also one of the key enzymes of nucleotide metabolism. XOD is mainly distributed in mammalian heart, lung, liver and other tissues. When liver function impaired, XOD is released into serum in a large amount, which has specific significance for the diagnosis of liver damage.

XOD catalyzes hypoxanthine to produce xanthine and superoxide anion. Superoxide anion reacts with hydroxylamine hydrochloride to form NO^{2-}, and the NO^{2-} under the action of p-aminobenzenesulfonamide and naphthalene ethylenediamine hydrochloride is produced a red azo compound with a characteristic absorption peak at 530 nm. The color depth is linear with XOD activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, constant temperature foster box/water-bath, centrifuge, micro glass cuvette/96-well flat-bottom plate, adjustable transferpettor, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation

1. Bacteria or cells: Collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. It is suggested that add 1 mL of Reagent I to 5 million of bacteria or cells. Use ultrasonication to split bacteria or cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 10 seconds,

repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing

2. Tissue: According to the proportion of tissue weight (g): Extract solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Reagent I and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing

3. Serum (plasma) sample: Detect directly. Centrifuge before detect if there are precipitation.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 530 nm and set spectrophotometer counter to zero with distilled water.
2. Standard working solution: dilute 10μmol/mL standard solution to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625μmol/mL with distilled water.
3. Operation table:

Reagent (μL)	Blank tube (Ab)	Test tube (At)	Standard tube (As)
Distilled water	10	-	-
Supernatant	-	10	-
Standard	-	-	10
Reagent I	40	40	40
Reagent III	40	40	40
Reagent IV	40	40	40
Mix and react for 20 min at 37℃			
Reagent V	60	60	60
Reagent VI	60	60	60
Mix and react for 20 min at 37℃. Measure the absorbance value at the wavelength of 530nm, and record them as Ab, At and As, and calculate ΔAt = At-Ab, ΔAs = As-Ab. Blank tube (Ab) and standard curve only be measured once or twice.			

III. Calculation:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔAt into the equation to get x (μmol/mL).

2. Calculation

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1nmol NO_2^- per minute every milligram protein.

XOD activity (U/mg prot) = $x \times 10^3 \div Cpr \div T \times F = x \times 50 \div Cpr \times F$

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1nmol NO_2^- per minute every gram tissue sample.

XOD activity (U/g weight) = $x \times 10^3 \div W \div T \times F = x \times 50 \div W \times F$

3) Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1nmol NO_2^- per minute every 10^4 bacteria or cells.

XOD activity (U/10^4 cell) = $x \times 10^3 \div 500 \div T \times F = x \times 0.1 \times F$

4) Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1nmol NO_2^- per minute every milliliter serum (plasma) sample.

XOD activity (U/mg prot) = $x \times 10^3 \div T \times F = x \times 50 \times F$

10^3: Unit conversion factor, 1 μmol=10^3 nmol;

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL;

500: Cells or bacteria, 5 million;

T: Reaction time, 20 minutes;

F: Dilution times.

Experimental examples:

1. Take 0.1011g rabbit kidney for sample processing and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate ΔAt = At-Ab=0.155-0.052= 0.103. Bring the result into the standard curve y=1.6275x - 0.0033, and calculate x=0.065. The result is calculated according to the sample weight:
   XOD activity (U/g weight) = $x \times 50 \div W$=32.302 U/g weight.
2. Take 0.01mL milk, dilute it with Reagent I 100 times and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate ΔAt = At-Ab=0.056-0.052= 0.004. Bring the result into the standard curve y=1.6275x - 0.0033, and calculate x=0.004. The result is calculated according to the sample weight.
   XOD activity (U/mL) = $x \times 50 \times F$=22.427 U/mL.

Related Products:

BC3590/BC3595	Hydrogen Peroxide (H_2O_2) Content Assay Kit
BC0690/BC0695	Glucose oxidase (GOD) Assay Kit
BC1270/BC1275	Protein Carbonyl Assay Kit
BC1280/BC1285	Diamine oxidase(DAO) Assay Kit