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Xanthine Oxidase(XOD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: AK0489

Size: 100T/96S

Product composition:

Extract solution: Liquid 110 mL×1, Storage at 4℃

Reagent Ⅰ: Liquid 20 mL×1, Storage at 4℃

Reagent II: Powder×1, Storage at 4℃

Product Description:

XOD (EC 1. 17.3.2) catalyzes the oxidation of xanthine to uric acid 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 the production of uric acid from jaundice, which has a characteristic absorption peak at 290 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader,centrifuge, adjustable pipette,micro quartz cuvette/ 96 well flat-bottom plate (UV), mortar/homogenizer, ice and distilled water.

Sample preparation:

A. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Suggested 5 million with 1mL of Extract solution. Use ultrasonic to splitting bacteria or cell (powder 20%, work time 3s， interval 10s， repeat for 30 times). centrifuge at 8000 g and 4℃for 10min. Supernatant is placed on ice for test.

B. Tissue

Suggested 0. 1g of tissue with 1mL of Extract solution. Fully grind on ice, centrifuge at 8000 g and 4℃for 10min. Supernatant is placed on ice for test.Or directly use 0.5mg/mL enzyme solution for direct measurement. To ensure the accuracy of the experiment, it is recommended to use a gradient dilution of the Extractsolution for determination.

C. Serum (plasma) sample

Detect sample directly.

Procedure:

1. Preheat spectrophotometer/microplate readerfor 30min, adjust the wavelength to 290 nm and set the counter to zero with distilled water.
2. Preparation of working solution: add 9.375 mL of Reagent I into each bottle of Reagent II when using, mix it well for use; the unused reagent can be stored for one week at 4℃, Dilute it with distilled water 10 times as needed.
3. Take a certain amount of working solution at 37℃ (mammal) or 25℃ (other species) water bath for 30 min before the measurement.
4. Blank tube: Take 0.25mL of working solution andadd 10μL of distilled water, immediately mix well and take out 200 μL to the micro quartz cuvette/96 well flat-bottom plate(UV), measure the initial absorbance value A1 at 290nm and the absorbance value A2 after 1min, calculate ΔA_B=A2 -A1.
5. Test tube: Take 0.25mL of working solution and add 10μL of distilled water, immediately mix well and take out 200 μL to the micro quartz cuvette/96 well flat-bottom plate(UV), immediately measure the initial absorbance value A1 at 290nm and the absorbance value A2 after 1min, calculate ΔA_T=A2 -A1.

Calculation:

A. micro quartz cuvette

1. XOD calculation of serum (plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of 1μmol of uric acid per minute every milliliter of serum.

Tyrosinase (U/mL)= $(\Delta A_T.\Delta A_B)\div(\varepsilon\times d)\times Vrv\times 10^6\div Vs\div T=2.131\times\Delta A$

2. XOD calculation in tissues, bacteria or cells:

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of 1μmol of uric acid per minute every milligram of tissue protein.

Tyrosinase (U/mg prot) =(ΔAT-ΔAB)÷(ε×d)×Vrv×106÷(Vs×Cpr)÷T=2. 131×ΔA÷Cpr。

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of uric acid per minute every gram of tissue.

Tyrosinase (U/g)= ΔA÷(ε×d)×Vrv×106÷(W÷Vsv×Vs)÷T=2. 131×ΔA÷W

(3) Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of uric acid per minute every 10^4 cell.

Tyrosinase (U/10^4 cell)=$\Delta A\div(\varepsilon\times d)\times Vrv\times 10^6\div(500\div Vsv\times Vs)\div T=4.262\times 10^{-3}\times\Delta A$

ε:molar extinction coefficient of uric acid, 1.22×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Vrv: total reaction volume, 2.6×10^{-4}L;

Vs: supernatant volume (mL), 0.01 mL;

Cpr: sample protein concentration (mg/mL);
T: Reaction time (min), 1 min;
W: Sample weight (g);
Vsv: Extraction volume, 1 mL;
500: 5 million cells.
10^9: 1 mol=10^6 μmol;

B. 96 well plate (UV)

3. XOD calculation of serum (plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of uric acid per minuteeverymilliliter of serum.

Tyrosinase (U/ml)= $(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times Vrv\times 10^6\div Vs\div T=3.552\times\Delta A$

4. XOD calculation in tissues, bacteria or cells:

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of 1μmol of uric acid per minuteevery milligram of tissue protein.

Tyrosinase (U/mg prot) =$(\Delta AT-\Delta AB)\div(\varepsilon\times d)\times Vrv\times 10^6\div(Vs\times Cpr)\div T=3.552\times\Delta A\div Cpr$。

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of 1μmol of uric acid per minuteevery gram of tissue.

Tyrosinase (U/g)= $\Delta A\div(\varepsilon\times d)\times Vrv\times 10^6\div(W\div Vsv\times Vs)\div T=3.552\times\Delta A\div W$

(3) Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of 1μmol of uric acid per minuteevery 10^4 cell.

Tyrosinase (U/10^4 cell)=$\Delta A\div(\varepsilon\times d)\times Vrv\times 10^6\div(500\div Vsv\times Vs)\div T=7.\ 103\times 10^{-3}\times\Delta A$

ε: molar extinction coefficient of uric acid, 1.22×10^4L/mol/cm;
d: light path of cuvette, 0.6cm;
Vrv: total reaction volume, 2.6×10^{-4}L;
Vs: supernatant volume (mL), 0.01 mL;
Cpr: sample protein concentration (mg/mL);
T: Reaction time (min), 1 min;
W: Sample weight (g);
Vsv: Extraction volume, 1 mL;
500: 5 million cells.
10^9: 1 mol=10^9 nmol;

Note:

1. when the Δ A_T greater than 0.4 or A3 is greater than 1.5 when suggest using extract dilution after samples are measured.

Experimental instances：

1. Take 0. 1g of rat liver, add 1mL of extract solution, fully grind on ice, centrifuge at 8000g for 10 min at 4℃ . Supernatant is placed on ice for test. Take the supernatant and detect according to the measured steps. CalculateΔA_B=A2-A1=0.3419-0.3351=0.007， ΔA_T= A4-A3=0.9970-0.9747=0.0223, calculate the enzyme activity according to sample weight:

XOD（U/g weight）=2. 131×ΔA÷W=0.326 U/g weight.

References：

[1] Zhao X, Zhu J X, Mo S F, et al. Effects of cassia oil on serum and hepatic uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver[J]. Journal of ethnopharmacology, 2006, 103(3): 357-365.

Related products：

AK0362/AK0361	Hydrogen Peroxide (H2O2) Content Assay Kit
AK0522/AK0521	Glucose oxidase (GOD) Assay Kit
AK0460/AK0459	Protein Carbonyl Assay Kit
AK0458/AK0457	Diamine oxidase(DAO) Assay Kit