Xanthine Oxidase (XOD) Activity Fluorometric Assay Kit

Catalog No: E-BC-F019

Method: Fluorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.01 U/L

Detection range: 0.01 -1.2 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can be used to measure Xanthine Oxidase (XOD) activity in serum, plasma, and animal tissue samples.

▲ Background

Xanthine oxidase (XOD) is widely distributed in the tissue and cell cytoplasm of human heart, lung, liver and so on, and the mucosa of small intestine is the most abundant. XOD in serum mainly comes from liver cells. When liver cells are damaged, the content of XOD in serum will increase sharply, which is of great significance for the identification of hepatocellular jaundice and obstructive jaundice. In addition, when XOD is abnormally active in the body, it will lead to the generation and excessive accumulation of a large amount of uric acid, leading to hyperuricemia and gout.

▲ Detection principle

Hypoxanthine are oxidized by xanthine oxidase (XOD) to produce xanthine and super oxygen anion, which will quickly converte to hydrogen peroxide in the system, and then, in the role of peroxidase, hydrogen peroxide can oxidize the non-fluorescent probe to fluorescent substance. By measuring the fluorescence value, the corresponding the activity of xanthine oxidase can be calculated.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20℃ , 6 months
Reagent 2	Probe Solution	0.3 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	-20℃ , 6 months
Reagent 4	Substrate	4 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 5	2 mmol/L H_2O_2 Standard Solution	1.5 mL × 1 vial	-20℃ , 6 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. It is recommended to aliquot the reagent 3 application solution into smaller quantities and store at -20℃ . Avoid repeated freeze-thaw cycles.
2. The reaction time should be accurate.
3. The sample size of each batch should be less than 20.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 application solution:
   Dissolve a vial of reagent 3 with 0.3 mL of reagent 1 and mix fully. Prepare the needed amount fresh solution before use. It can be stored at -20℃ for 30 days.
   Note: It is recommended to aliquot the reagent 3 application solution into smaller quantities and store at -20℃ . Avoid repeated freeze-thaw cycles.
2. Preparation of sample working solution:
   Mix the reagent 4, reagent 2 and reagent 3 application solution at a ratio of 23:1:1. Prepare the needed amount fresh solution before use and store with shading light.
3. Preparation of control working solution:
   Mix the reagent 1, reagent 2 and reagent 3 application solution at a ratio of 23:1:1. Prepare the needed amount fresh solution before use and store with shading light.
4. Preparation of 20 μmol/L H_2O_2 standard solution:
   Mix the reagent 5 and double distilled water at a ratio of 1:99. Prepare the needed amount fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01 -1.2 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Mouse serum	1
Dog serum	3-5
Rat plasma	1
10% Rat kidney tissue homogenate	5-10
Horse serum	1
Human plasma	1
10% Mouse heart tissue homogenate	5-10
10% Rat lung tissue homogenate	5-10

Note: The diluent is reagent 1.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	Ex/Em=535 nm/587 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

▲Operating steps

1. The preparation of standard curve

Dilute 20 μmol/L H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 6, 8, 10, 12 μmol/L.

2. The measurement of samples

1) Standard well: Add 50 μL of standard with different concentrations into the well.

Sample well: Add 50 μL of sample into the well.

Control well: Add 50 μL of sample into the well.

2) Add 50 μL of sample working solution into standard and sample well.
Add 50 μL of control working solution into control well.

3) Mix fully with microplate reader for 5 s and stand at room temperature for 2 min.

4) Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as F_1, and then stand at room temperature with shading light for 10 min, under the same wavelength conditions to determine the fluorescence value of each well, recorded as F_2, $F_{sample}=F_{2\ (sample)}-F_{1\ (sample)}$, $F_{control}=F_{2\ (control)}-F_{1\ (control)}$. (Note: There was no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_{2(standard)}$.)

▲Operation table

	Standard well	Sample well	Control well
Standard with different concentrations (μL)	50		
Samples (μL)		50	50
Sample working solution (μL)	50	50	
Control working solution (μL)			50
Mix fully with microplate reader for 5 s and stand at room temperature for 2 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as F_1, and then stand at room temperature with shading light for 10 min, under the same wavelength conditions to determine the fluorescence value of each hole, recorded as F_2, $F_{sample}=F_{2\ (sample)}-F_{1\ (sample)}$, $F_{control}=F_{2\ (control)}-F_{1\ (control)}$. (Note: There was no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_{2(standard)}$.			

Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

Definition: The amount of XOD in 1 L of serum or plasma that catalyze the production of 1 μmol H_2O_2 per minute at 25℃ is defined as 1 unit.

$$\text{XOD activity (U/L)} = (\Delta F - b) \div a \div T \times f$$

2. Tissue sample:

Definition: The amount of XOD in 1 g of tissue protein that catalyze the production of 1 μmol H_2O_2 per minute at 25℃ is defined as 1 unit.

$$\text{XOD activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

Note:

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank.}$ (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: The absolute fluorescence value of sample, $F_{Sample} - F_{Control.}$

T: the reaction time, 10 min.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.01 -1.2 U/L	Average inter-assay CV (%)	4.1
Sensitivity	0.01 U/L	Average inter-assay CV (%)	9.0

▲Example analysis

Dilute rat lung tissue, dilute 10% rat lung tissue homogenate with reagent 1 for 10 times, add 50 μL of diluted sample to the well and carry the assay according to the operation table. The results are as follows:

standard curve: y=591.94 x - 163.41, the average $F_{1\ (sample)}$ value of the sample is 1847.38, the average $F_{2(sample)}$ value of the sample is 4865.6, F_{sample}=4865.6 – 1847.38 = 3018.22; the average $F_{1\ (control)}$ value of the sample is 408.91, the average $F_{2(control)}$ value of the sample is 475.04, $F_{control}$=475.04 - 408.91 = 66.13; $\Delta F = F_{Sample} - F_{Control}$ = 3018.22 – 66.13 = 2952.09, the concentration of protein in sample is 3.94 gprot/L, and the calculation result is:

$$\text{XOD activity (U/gprot)} = \frac{(2952.09+163.41)}{591.94} \div 10 \times 10 \div 3.94 = 1.34\ \text{U/gprot}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

▲ Plasma

The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

▲ Tissue sample

Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note:

1. Homogenized medium: Reagent 1.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.