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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K805-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（540-560 nm）

Detection range：0.067-39.30 U/L

Elabscience® Xanthine Oxidase (XOD) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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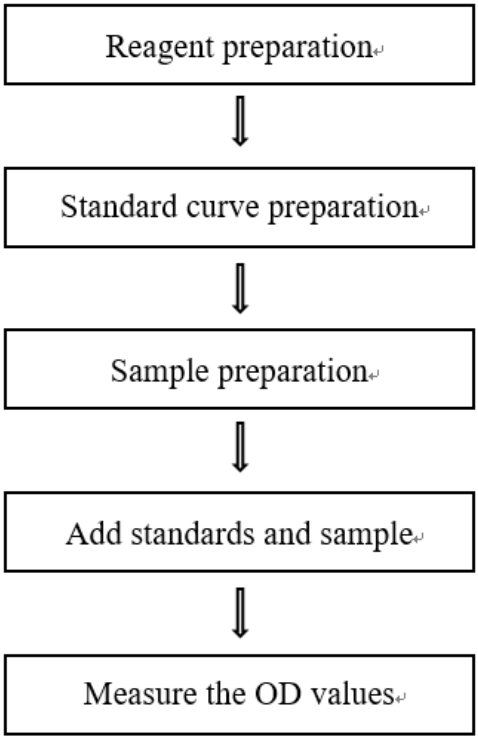
Assay summary



Intended use

This kit can measure xanthine oxidase (XOD) activity in serum, plasma and animal tissue samples.

Detection principle

Xanthine Oxidase (XOD) is mainly present in milk, liver and spleen of mammals, belonging to aerobic dehydrogenases, which is an important enzyme in nucleic acid metabolism in the body. When hepatocytes are injured, this enzyme is released into the serum earlier than SGPT, and increase its activity in serum significantly, which has obvious significance for the identification of hepatocellular jaundice and it is used for the identification of obstructive jaundice. In the process of hypoxia, xanthine dehydrogenase quickly forms xanthine oxidase, which plays an important role in free radical production.

XOD can catalyze hypoxanthine to xanthine, and at the same time while producing superoxide anion free radicals. In the presence of electron acceptor and chromogenic agent, the purplish red substance can be generated, and the vitality of XOD can be calculated by measuring the production of purplish red substance.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	20 mL ×1 vial	-20℃, 12 months
Reagent 2	Substrate Solution	0.5 mL ×1 vial	1 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Enzymatic Reagent	0.5 mL ×1 vial	1 mL ×1 vial	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent 1	0.8 mL ×1 vial	1.6 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent 2	0.8 mL ×1 vial	1.6 mL ×1 vial	-20℃, 12 months, shading light
Reagent 6	1 mmol/L Standard Solution	1.6 mL ×1 vial	3.2 mL ×1 vial	-20℃, 12 months
	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (540-560 nm, optimum wavelength: 550 nm), Incubator(37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of chromogenic working solution:

Dliute 20 μL of chromogenic agent 1 with 20 μL of chromogenic agent 2, mix well. The chromogenic working solution should be prepared on spot. The prepared solution should be stored with shading light and used up within 1 hour.

③ Preparation of working solution:

For each well, prepare 180 μL of working solution (mix well 147 μL of buffer solution, 6.5 μL of substrate solution, 6.5 μL of enzymatic reagent and 20 μL of chromogenic working solution). The working solution should be prepared on spot and stored protected from light.

④ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1**
1 mmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	160	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
Rat plasma	1
Rat serum	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.

Sample well: Add 20 μL of sample into the corresponding well.

② Add 180 μL of working solution into each well.

③ Mix fully with microplate reader for 5 s, measure the OD values of sample well at 550 nm with microplate reader, recorded as A_1.

④ Incubation at 37℃ for 25 min, measure the OD values of sample well and standard well, recorded as A_2.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. For serum, plasma sample:

Definition: The amount of xanthine oxidase (XOD) in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol H2O2 in 1 minute at 37°C is defined as 1 unit.

$$\text{XOD activity (U/L)} = (\Delta A_{550} - b) \div a \div T \times f \times 1000$$

2. Tissue sample:

Definition: The amount of xanthine oxidase (XOD) in 1 g tissue that hydrolyze the substrate to produce 1 μmol H2O2 in 1 minute at 37°C is defined as 1 unit.

$$\text{XOD activity (U/gprot)} = (\Delta A_{550} - b) \div a \div T \times f \div C_{pr} \times 1000$$

[Note]

ΔA_{550}: The change OD of the sample ($\Delta A_{550} = A_2 - A_1$).

T: The time of reaction, 25 min.

f: Dilution factor of sample before tested.

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

1000： 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.20	13.50	26.40
%CV	3.4	3.0	2.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.20	13.50	26.40
%CV	9.0	10.4	10.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.46	0.77
Observed Conc. (mmol/L)	0.2	0.4	0.9
Recovery rate (%)	89	93	115

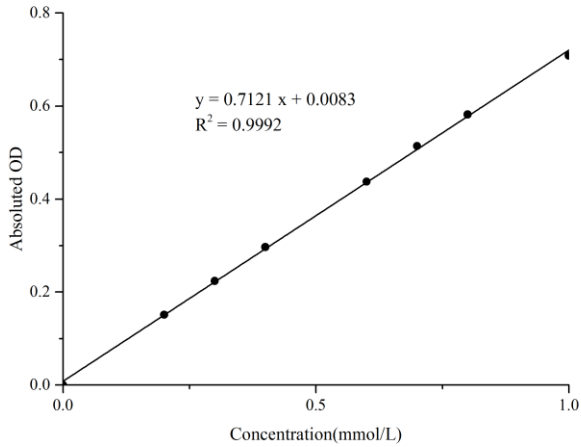
Sensitivity

The analytical sensitivity of the assay is 0.067 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1.0**
OD value	0.042	0.194	0.268	0.327	0.484	0.561	0.631	0.757
	0.041	0.192	0.263	0.350	0.474	0.550	0.616	0.743
Average OD	0.042	0.193	0.266	0.339	0.479	0.556	0.624	0.750
Absoluted OD	0	0.152	0.224	0.297	0.438	0.514	0.582	0.709



Appendix II Example Analysis

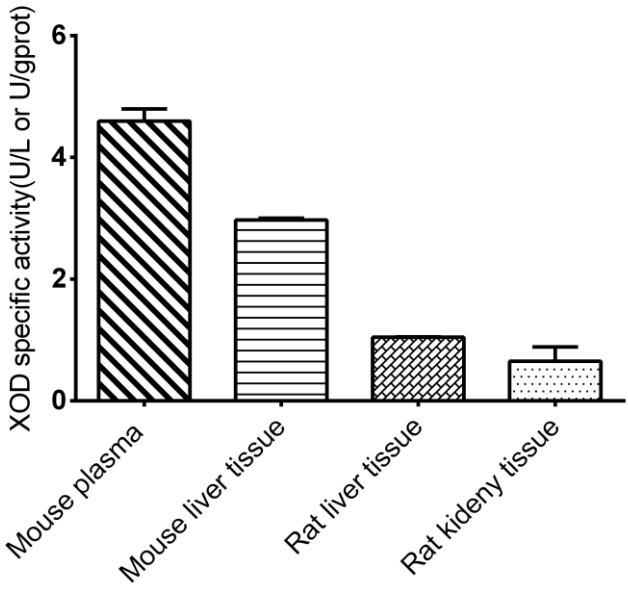
Example analysis:

Take 20 μL of 10% Mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.7121 x + 0.0083, the A1 of the sample is 0.144, incubate at 37°C for 25 min, the A2 of the sample is 0.453, $\Delta A_{550} = A_2 - A_1 = 0.453 - 0.144 = 0.309$, the concentration of protein in sample is 5.68 gprot/L, and the calculation result is:

XOD activity (U/gprot)=(0.309 - 0.0083) ÷ 0.7121 ÷ 25 ÷ 5.68 × 1000 = 2.97 U/gprot

Detect Mouse plasma, 10% Mouse liver tissue homogenate (the concentration of protein is 5.68 gprot/L), 10% Rat liver tissue homogenate (the concentration of protein is 5.01 gprot/L), 10% Rat kideny tissue homogenate (the concentration of protein is 4.19 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.