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

Catalog No：E-BC-F019

Specification：96T(40 samples) /500Assays(242 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.01 -1.2 U/L

Elabscience® Xanthine Oxidase (XOD) Activity Fluorometric 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
Tel: 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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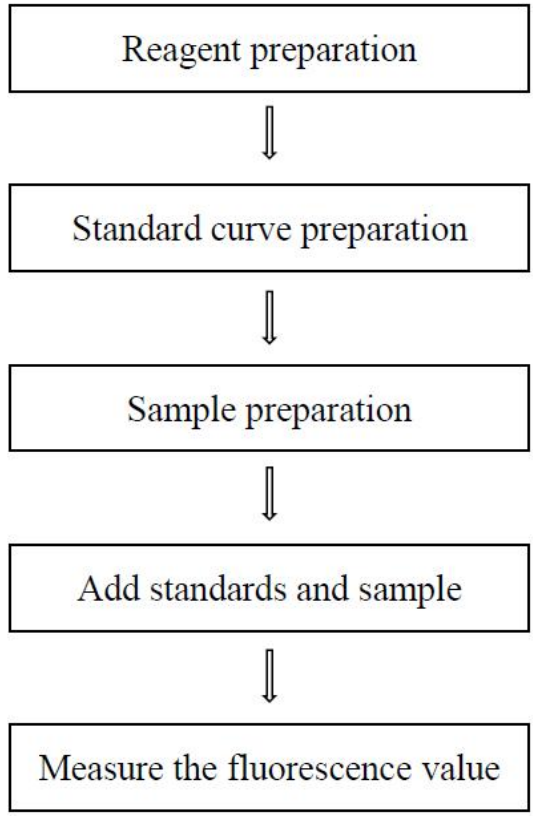
Assay summary



Intended use

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

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	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 5 vials	-20°C, 12 months
Reagent 2	Probe Solution	0.3 mL × 1 vial	1.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	Powder × 5 vials	-20°C, 12 months
Reagent 4	Substrate	4 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	2 mmol/L H_2O_2 Standard Solution	1.5 mL × 1 vial	7 mL × 1 vial	-20°C, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme application solution:
Dissolve one vial of enzyme with 300 μL of buffer solution, Mix well to dissolve. Store at -20°C for 30 days.
Note: Aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of sample working solution:
For each well, prepare 50 μL of sample working solution (mix well 46 μL of substrate, 2 μL of probe solution and 2 μL of enzyme application solution). The sample working solution should be prepared on spot and protected from light.

④ The preparation of control working solution:
For each well, prepare 50 μL of control working solution (mix well 46 μL of buffer solution, 2 μL of probe solution and 2 μL of enzyme application solution). The control working solution should be prepared on spot and protected from light.

⑤ The preparation of 20 μmol/L H_2O_2 standard solution:
Add 5 μL of 2 mmol/L H_2O_2 standard solution and 495 uL of double distilled water, mix well. The 20 μmol/L H_2O_2 standard solution should be prepared on spot.

⑥ The preparation of standard curve：

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

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1**	**2**	**4**	**6**	**8**	**10**	**12**
20 μmol/L standard (μL)	0	10	20	40	60	80	100	120
Double distilled water (μL)	200	190	180	160	140	120	100	80

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°C 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 buffer solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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
Mouse serum	1
Dog serum	3-5
Rat plasma	1
Horse serum	1
Human plasma	1
10% Rat kidney tissue homogenate	5-10
10% Mouse heart tissue homogenate	5-10
10% Rat lung tissue homogenate	5-10

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① It is recommended to aliquot the enzyme application solution and store at -20°C. Avoid repeated freeze/thaw cycles is advised.

② The reaction time should be accurate.

③ The sample size of each batch should be less than 20.

Operating steps

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 $F_{2(standard)}$.)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value ($F_{2(standard)}$) of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value ($\Delta F_{2(standard)}$) 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. 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°C 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°C is defined as 1 unit.

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

[Note]

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.

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)	0.10	0.50	1.00
%CV	4.8	4.2	3.3

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)	0.10	0.50	1.00
%CV	8.5	10.0	8.5

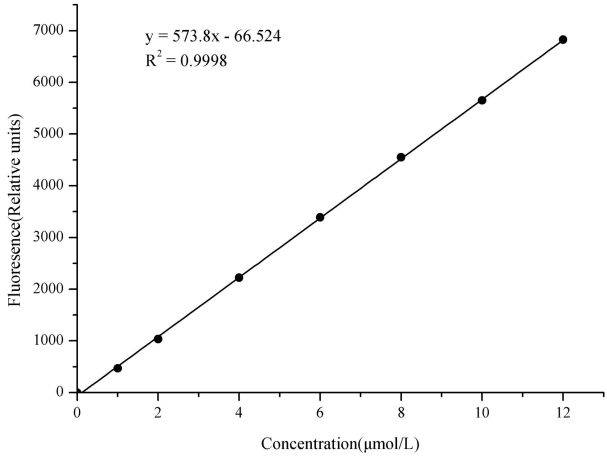
Sensitivity

The analytical sensitivity of the assay is 0.01 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 fluorescence 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 (μmol/L)	**0**	**1**	**2**	**4**	**6**	**8**	**10**	**12**
Fluorescence value	156	616	1161	2311	3506	4722	5740	7092
	157	634	1224	2443	3581	4693	5874	6873
Average fluorescence value	156	625	1193	2377	3543	4707	5807	6982
Absoluted fluorescence value	0	469	1036	2221	3387	4551	5651	6826



Appendix II Example Analysis

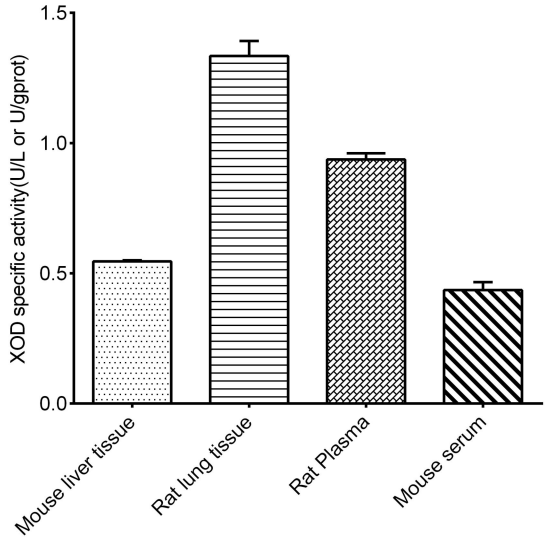
Example analysis：

Dilute rat lung tissue with buffer solution for 10 times, add 50 μL of diluted sample to the well and carry the assay according to the operation steps. 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; ΔF =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:

XOD activity (U/gprot) = (2952.09 + 163.41) ÷ 591.94 ÷ 10 × 10 ÷ 3.94 = 1.34 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 8.64 gprot/L, dilute for 10 times), 10% rat lung tissue homogenate (the concentration of protein is 3.94 gprot/L, dilute for 10 times), rat plasma and mouse serum 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.