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

Catalog No：E-BC-F024

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.10-50 μmol/L

Elabscience® Xanthine/Hypoxanthine 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
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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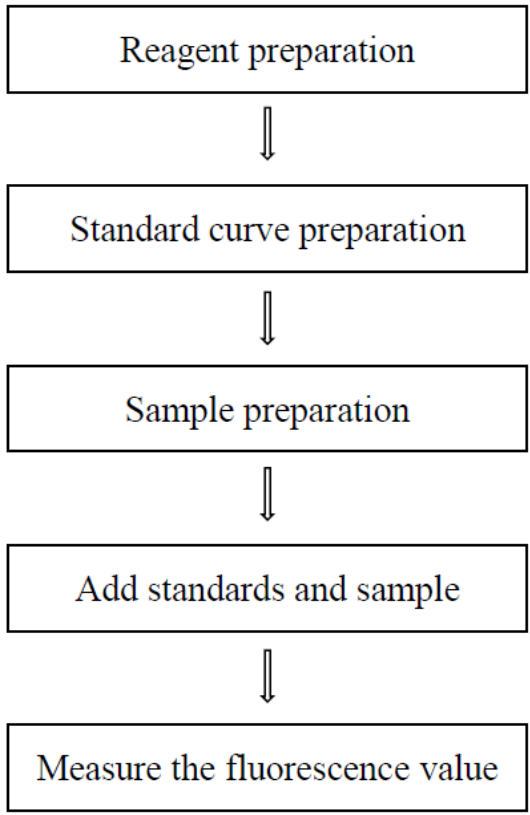
Assay summary



Intended use

This kit can be used to measure xanthine/hypoxanthine content in serum (plasma), animal tissue and cell samples.

Detection principle

Xanthine and hypoxanthine are important alkaloid purines that can be used to treat symptoms such as asthma relief and bronchodilation. Hypoxanthine can be oxidized by xanthine oxidase to form xanthine. Xanthine is catalyzed by xanthine oxidase to form uric acid. When xanthine oxidase is deficient, xanthine accumulates in urine and blood, inducing hyperuricemia and gout, and eventually leading to renal failure.

The detection principle of this kit: Xanthine/hypoxanthine generates intermediate products under the catalysis of enzymes. Under the action of other enzymes, the intermediate products react with the probe to form fluorescent substances. By substituting the fluorescence values into the standard curve, the total content of xanthine and hypoxanthine in the sample can be calculated.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL×1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Enzyme Reagent	Powder×1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 3	Probe	0.06 mL×1 vial	0.12 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	10 mmol/L Standard	0.2 mL×1 vial	0.4 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	48 wells	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), Incubator

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

10 kDa MWCO Spin Filter（Outer tube 1.5 mL, Inter tube 0.5 mL）

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 550 μL of double distilled water. Mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution. For example, prepare 1910 μL of reaction working solution (mix well 1800 μL of buffer solution, 100 μL of enzyme working solution and 10 μL of probe). The reaction working solution should be prepared on spot protected from light and used up within 30 min.

④ The preparation of 50 μmol/L standard solution:

Before testing, please prepare sufficient 50 μmol/L standard solution. For example, prepare 2000 μL of 50 μmol/L standard solution (mix well 1990 μL of buffer solution and 10 μL of 10 mmol/L standard). The 50 μmol/L standard solution should be prepared on spot protected from light and used up within 8 h.

⑤ The preparation of standard curve:

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

Dilute 50 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 2.5, 5, 10, 20, 30, 40, 50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2.5**	**5**	**10**	**20**	**30**	**40**	**50**
50 μmol/L Standard (μL)	0	10	20	40	80	120	160	200
Buffer Solution (μL)	200	190	180	160	120	80	40	0

Sample preparation

① Sample preparation

Serum or plasma samples: Add serum (plasma) sample into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 25 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Homogenize 50 mg tissue in 450 µL buffer solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and add it into 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection.

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 µL buffer solution with a dounce homogenizer.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and add it into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 25 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection.

② **Dilution of sample**

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

Sample type	Dilution factor
Human serum	2-20
Rat serum	1
10% Rat kidney tissue homogenate	5-20
10% Rat brain tissue homogenate	2-10
10% Rat lung tissue homogenate	1
10% Rat liver tissue homogenate	5-10
10% Rat heart tissue homogenate	8-20
10% Rat spleen tissue homogenate	8-20
1×10^6 HL-60 cells	1
1×10^6 293T cells	1

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

Operating steps

① Standard well: add 10 μL of standard solution with different concentrations into the wells.
Sample well: add 10 μL of sample into the wells.

② Add 190 μL of reaction solution into each well.

③ Mix fully with fluorescence microplate for 5s. Incubate at 37°C for 30 min.Measure the fluorescence at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value 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 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) samples:

$$\text{Xanthine/hypoxanthine content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{Xanthine/hypoxanthine content (μmol/kg wet weight)} = (\Delta F - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\text{Xanthine/hypoxanthine content (nmol/10^6)} = (\Delta F - b) \div a \times V \div n \times f \times 1000$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{blank}$ (The absolute fluorescence value of sample well).

m: The weight of tissue, kg.

V: The volume of buffer solution, L.

n: The number of cell sample/10^6.

f: Dilution factor of sample before tested.

1000: 1 μmol/L = 1000 nmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.0	25.0	35.0
%CV	0.4	1.2	3.2

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.0	25.0	35.0
%CV	2.9	7.4	8.3

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 100.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc(μmol/L)	15.0	25.0	35.0
Observed Conc(μmol/L)	14.55	24.90	36.75
Recovery rate (%)	97	100	105

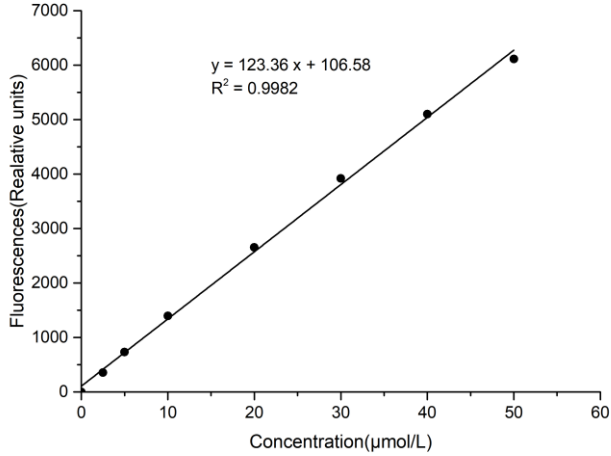
Sensitivity

The analytical sensitivity of the assay is 0.10 μmol/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	2.5	5	10	20	30	40	50
Fluorescence value	813	1168	1532	2185	3432	4668	5930	6954
	821	1175	1570	2245	3514	4812	5907	6908
Average fluorescence value	817	1172	1551	2215	3473	4740	5919	6931
Absoluted fluorescence value	0	355	734	1398	2656	3923	5102	6114



Appendix Π Example Analysis

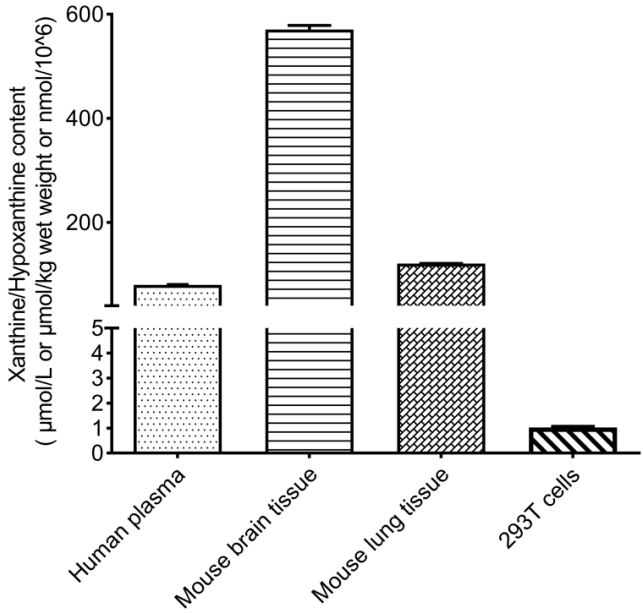
Example analysis：

Take 10 μL of 10% mouse brain tissue filtrate which dilute for 2 times and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 147.48 x - 192.55, the average fluorescence value of the blank well is 683, the average fluorescence value of the sample well is 5213, $\Delta F = F_{sample} - F_{blank}$ = 5213 - 683 = 4530, and the calculation result is:

Xanthine/hypoxanthine content (μmol/kg wet weight) = (4530 + 192.55) ÷ 147.48 ×

0.0009 ÷ 0.0001 ×2 = 576.39 μmol/kg wet weight

Detect human serum (dilute for 2 times), 10% mouse brain tissue homogenate supernatant (dilute for 2 times), 10% mouse lung tissue homogenate supernatant (dilute for 2 times) and 1×10^6 293T cells 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.