TECHNICAL MANUAL

Xanthine Oxidase (XOD) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0006
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure fluorescence intensity

2. Intended use

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

3. Detection principle

Hypoxanthine is oxidized by xanthine oxidase (XOD) to produce xanthine and superoxide anion, which quickly converts to hydrogen peroxide in the system. Subsequently, in the presence of peroxidase, hydrogen peroxide oxidizes the non-fluorescent probe to a fluorescent substance. By measuring the fluorescence value, the corresponding activity of xanthine oxidase can be calculated.

4. 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, protect from 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, protect from light
Reagent 5	2 mmol/L H2O2 Standard Solution	1.5 mL × 1 vial	7 mL × 1 vial	-20°C, 12 months
	Black Microplate	96 wells	96 wells	No requirement

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme with 300 µL of buffer solution. Mix well to dissolve. Store at -20°C for 30 days. Note: Aliquot storage at -20°C and avoid repeated freeze/thaw cycles.
3. **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 working solution). The sample working solution should be prepared fresh and protected from light.
4. **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 working solution). The control working solution should be prepared fresh and protected from light.
5. **Preparation of 20 µmol/L H2O2 standard solution:** Add 5 µL of 2 mmol/L H2O2 standard solution and 495 µL of double distilled water, mix well. The 20 µmol/L H2O2 standard solution should be prepared fresh.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 20 µmol/L H2O2 standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 1, 2, 4, 6, 8, 10, 12 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one 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 10,000×g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

2. The reaction time should be accurate.

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

9. 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 wells. 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 excitation wavelength 535 nm and emission wavelength 587 nm, recorded as F1, then stand at room temperature protected from light for 10 min. Under the same wavelength conditions, determine the fluorescence value of each well, recorded as F2. F_sample = F2(sample) - F1(sample), F_control = F2(control) - F1(control). Note: There was no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of F2(standard).

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value (F2(standard)) of the blank (Standard #1) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value (deltaF2(standard)) of standard and corresponding 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 catalyzes the production of 1 μmol H2O2 per minute at 25°C is defined as 1 unit.

XOD activity (U/L) = (deltaF - b) ÷ a ÷ T × f

2. Tissue sample:

Definition: The amount of XOD in 1 g of tissue protein that catalyzes the production of 1 μmol H2O2 per minute at 25°C is defined as 1 unit.

XOD activity (U/gprot) = (deltaF - b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

deltaF: 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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

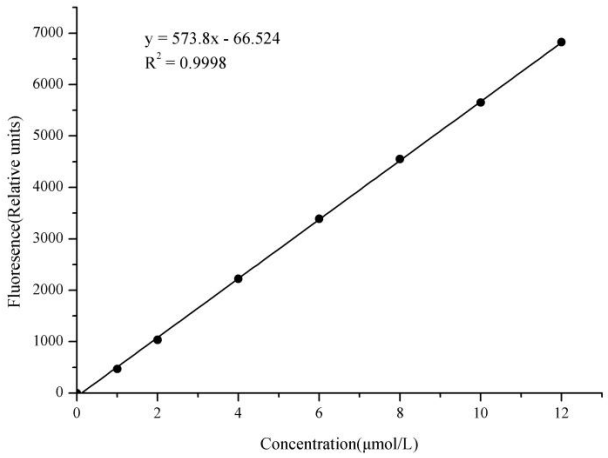
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.10	0.50	1.00
%CV	8.5	10.0	8.5

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	156	157	156	0
1	616	634	625	469
2	1161	1224	1193	1036
4	2311	2443	2377	2221
6	3506	3581	3543	3387
8	4722	4693	4707	4551
10	5740	5874	5807	5651
12	7092	6873	6982	6826

12. Appendix II Example Analysis

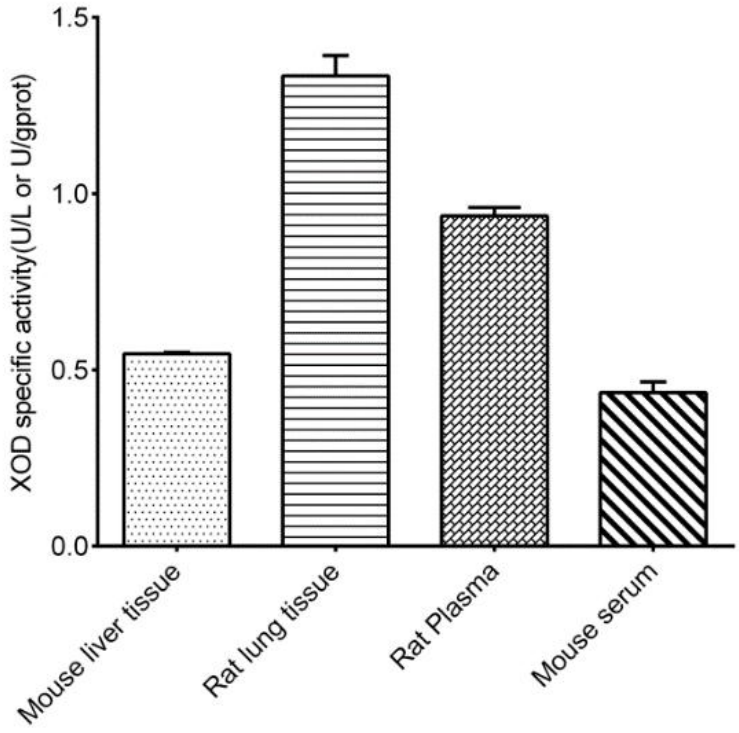
Example analysis:

Dilute rat lung tissue with buffer solution 10-fold, add 50 μL of diluted sample to the well and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = 591.94x - 163.41, the average F1(sample) value of the sample is 1847.38, the average F2(sample) value of the sample is 4865.6, F_{sample} = 4865.6 - 1847.38 = 3018.22; the average F1(control) value of the sample is 408.91, the average F2(control) value of the sample is 475.04, $F_{control}$ = 475.04 - 408.91 = 66.13; deltaF = 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 (protein concentration 8.64 gprot/L, diluted 10-fold), 10% rat lung tissue homogenate (protein concentration 3.94 gprot/L, diluted 10-fold), rat plasma and mouse serum according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.