# TECHNICAL MANUAL

# Xanthine Oxidase (XOD) Activity Assay Kit

- **SKU CODE:** MAES0265
- **SIZE:** 48 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 OD values

# 2. Intended use

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

# 3. 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 increases its activity in serum significantly, which has obvious significance for the identification of hepatocellular jaundice and 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 produce superoxide anion free radicals. In the presence of electron acceptor and chromogenic agent, a purplish red substance can be generated, and the activity of XOD can be calculated by measuring the production of purplish red substance.

# 4. Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Buffer Solution | 10 mL x 1 vial | 20 mL x 1 vial | -20 °C, 12 months |
| Reagent 2 | Substrate Solution | 0.5 mL x 1 vial | 1 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 3 | Enzymatic Reagent | 0.5 mL x 1 vial | 1 mL x 1 vial | -20 °C, 12 months, shading light |

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 4 | Chromogenic Agent 1 | 0.8 mL x 1 vial | 1.6 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 5 | Chromogenic Agent 2 | 0.8 mL x 1 vial | 1.6 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 6 | 1 mmol/L Standard Solution | 1.6 mL x 1 vial | 3.2 mL x 1 vial | -20 °C, 12 months |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | 2 pieces | |

# 5. Materials prepared by users

**Instruments:**

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

**Reagents:**

Normal saline (0.9% NaCl)

# 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** Dilute 20 µL of chromogenic agent 1 with 20 µL of chromogenic agent 2, mix well. The chromogenic working solution should be prepared immediately before use. The prepared solution should be stored protected from light and used 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 immediately before use and stored protected from light.
4. **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.

# 7. 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 normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C. 4. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 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 perform a pretest to confirm the dilution factor.

# 8. 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 A1.
4. Incubate at 37 °C for 25 min, measure the OD values of sample well and standard well, recorded as A2.

# 9. 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 absolute OD value.

3. Plot the standard curve by using absolute 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 hydrolyzes the substrate to produce 1 μmol H2O2 in 1 minute at 37 °C is defined as 1 unit.

XOD activity (U/L) = (deltaA$_{550}$ - b) ÷ a ÷ T × f × 1000

2. Tissue sample:

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

XOD activity (U/gprot) = (deltaA$_{550}$ - b) ÷ a ÷ T × f ÷ C$_{pr}$ × 1000

[Note]

deltaA$_{550}$: The change OD of the sample (deltaA$_{550}$ = 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.

# 10. 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) | 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.

## Inter-assay Precision

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

## Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration performed 6 times in parallel to get the average recovery rate of 99%.

## Recovery

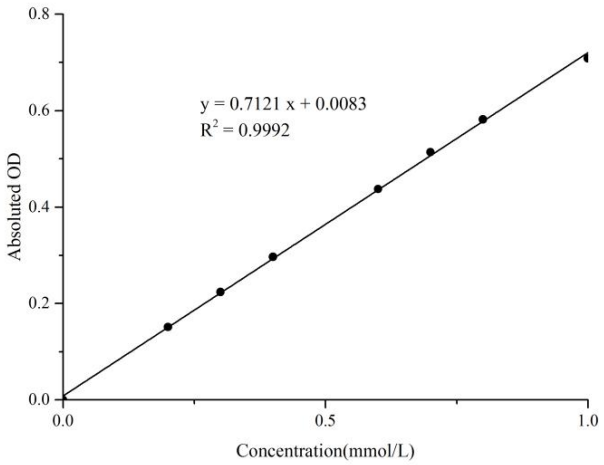
| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Expected Conc. (mmol/L) | 0.25 | 0.46 | 0.77 |
| Observed Conc. (mmol/L) | 0.2 | 0.4 | 0.9 |
| Recovery rate (%) | 89 | 93 | 115 |

## 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.

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




## Standard curve data

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

# 11. Appendix II Example Analysis

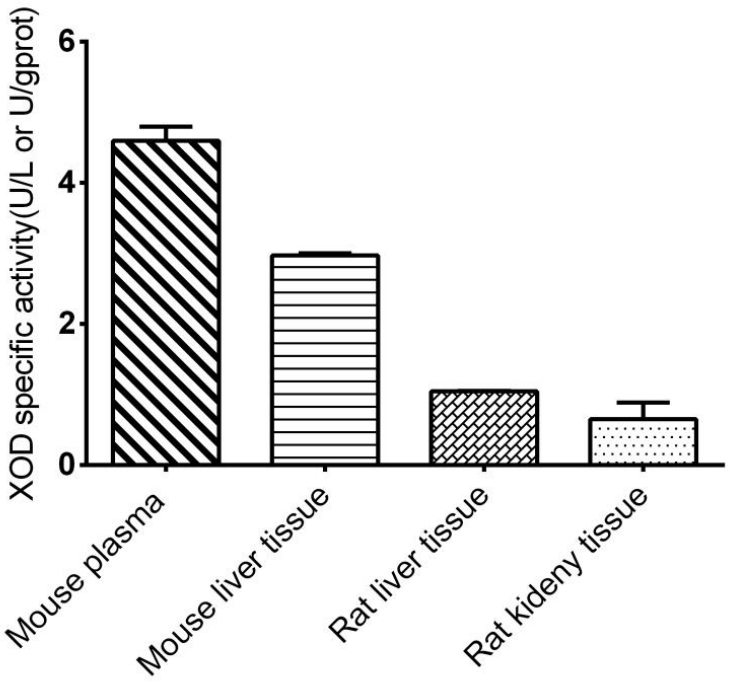
**Example analysis:**

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

**Standard curve:** y = 0.7121 x + 0.0083, the $A_1$ of the sample is 0.144, incubate at 37 °C for 25 min, the $A_2$ 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

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 kidney tissue homogenate (the concentration of protein is 4.19 gprot/L) were detected according to the protocol, the result is as follows:




# 12. 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 is not within 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.**