# Technical Manual

# Xanthine Oxidase (XOD) Fluorometric Activity Assay Kit

- Catalogue Code: MAES0006
- Size: 96T
- Research Use Only

# 1. Key features and Sample Types

## Detection method:

Fluorimetric method

## Specification:

96T

## Range:

0.01 -1.2 U/L

## Sensitivity:

0.01 U/L

## Storage:

-20°C for 6 months

## Expiry:

See Kit Label

## Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

## 2. Background

Xanthine oxidase (XOD) is widely distributed in the tissue and cell cytoplasm of human heart, lung, liver and so on, and the mucosa of small intestine is the most abundant. XOD in serum mainly comes from liver cells. When liver cells are damaged, the content of XOD in serum will increase sharply, which is of great significance for the identification of hepatocellular jaundice and obstructive jaundice. In addition, when XOD is abnormally active in the body, it will lead to the generation and excessive accumulation of a large amount of uric acid, leading to hyperuricemia and gout.

## 3. Intended Use

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

## 4. 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.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Buffer Solution** | 60 mL × 1 vial | -20°C, 6 months |
| **Probe Solution** | 0.3 mL × 1 vial | -20°C, 6 months, avoid direct sunlight |
| **Enzyme Reagent** | Lyophilized ×1 vial | -20°C, 6 months |
| **Substrate** | 4 mL × 1 vial | -20°C, 6 months, avoid direct sunlight |
| **2 mmol/L $H_2O_2$ Standard Solution** | 1.5 mL × 1 vial | -20°C, 6 months |
| **Black Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

## 6. Assay Notes:

1. Dilute the samples to the optimal concentration for detection if the XOD activity of samples exceeds the detection range.

2. The reaction time should be accurate.

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

## 7. Reagent Preparation

1. Preparation of **enzyme application solution:** Dissolve a vial of enzyme reagent with 0.3 mL of buffer solution and mix fully. Prepare the required amount of fresh solution before use. It can be stored at -20°C for 30 days.
   **Note:** It is recommended to aliquot enzyme application solution into smaller quantities and store at -20°C. Avoid repeated freeze-thaw cycles.

2. Preparation of **sample working solution:** Mix the substrate, probe solution and enzyme application solution at a ratio of 46:2:2. Prepare the required amount of fresh solution before use and avoid direct sunlight.

3. Preparation of **control working solution:** Mix the buffer solution, probe solution and enzyme application solution at a ratio of 46:2:2. Prepare the required amount of fresh solution before use and avoid direct sunlight.

4. Preparation of **20 μmol/L $H_2O_2$ standard solution:** Mix $H_2O_2$ standard solution (2 mmol/L) and double distilled water at a ratio of 1:99. Prepare the required amount of fresh solution before use.

## 8. Sample Preparation

**1. Serum sample:**
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

**2. Plasma sample:**
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

**3. Tissue sample:**
Take 0.02-1g fresh tissue and wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of buffer solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

## Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

## Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01 -1.2 U/L).

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 |
| 10% Rat kidney tissue homogenate | 5-10 |
| Horse serum | 1 |
| Human plasma | 1 |
| 10% Mouse heart tissue homogenate | 5-10 |
| 10% Rat lung tissue homogenate | 5-10 |

**Note:** The diluent is buffer solution.

# 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** Ex/Em=535 nm/587 nm

### Plate Set Up:

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | A | A | S1 | S1' | S9 | S9' | S17 | S17' | S25 | S25' | S33 | S33' |
| **B** | B | B | S2 | S2' | S10 | S10' | S18 | S18' | S26 | S26' | S34 | S34' |
| **C** | C | C | S3 | S3' | S11 | S11' | S19 | S19' | S27 | S27' | S35 | S35' |
| **D** | D | D | S4 | S4' | S12 | S12' | S20 | S20' | S28 | S28' | S36 | S36' |
| **E** | E | E | S5 | S5' | S13 | S13' | S21 | S21' | S29 | S29' | S37 | S37' |
| **F** | F | F | S6 | S6' | S14 | S14' | S22 | S22' | S30 | S30' | S38 | S38' |
| **G** | G | G | S7 | S7' | S15 | S15' | S23 | S23' | S31 | S31' | S39 | S39' |
| **H** | H | H | S8 | S8' | S16 | S16' | S24 | S24' | S32 | S32' | S40 | S40' |

**Note:** A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

# 10. Operation Steps

### The preparation of standard curve

Dilute $H_2O_2$ standard solution (20 μmol/L) 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.

### The measurement of samples

1. **Standard well:** Add 50 μL of standard with different concentrations into the well.
   **Sample well:** Add 50 μL of sample into the wells.
   **Control well:** Add 50 μL of sample into the wells.

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 5s 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 avoid direct sunlight 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 $F_{2(standard)}$.).



**Email:** info@assaygenie.com **Web:** www.assaygenie.com

### Operation Table

| | Standard well | Sample well | Control well |
|---|---|---|---|
| **Standard with different concentrations (μL)** | 50 | | |
| **Samples (μL)** | | 50 | 50 |
| **Sample working solution (μL)** | 50 | 50 | |
| **Control working solution (μL)** | | | 50 |
| Mix fully with microplate reader for 5s and stand at room temperature for 2 min. 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 avoid direct sunlight for 10 min, under the same wavelength conditions to determine the fluorescence value of each hole, 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 $F_{2(standard)}$. | | | |

## 11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

**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℃ is defined as 1 unit.

XOD activity (U/L) = (ΔF - b) ÷ a ÷ T × 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℃ is defined as 1 unit.

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

**y:** The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ ($F_{Blank}$ is the F value when the standard concentration is 0)

**x:** The concentration of standard

**a:** The slope of standard curve

**b:** The intercept of standard curve

**ΔF:** The absolute fluorescence value of sample, $F_{Sample} - F_{Control}$

**T:** the reaction time, 10 min

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

**f:** Dilution factor of sample before tested

## 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.01 -1.2 U/L |
| **Sensitivity** | 0.01 U/L |
| **Average inter-assay CV (%)** | 9.0 |
| **Average intra-assay CV (%)** | 4.1 |

### Analysis

Dilute rat lung tissue with buffer solution for 10 times, add 50 μL of diluted sample to the well, carry the assay according to the operation table.

**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:

$$\boldsymbol{XOD\ activity}\left(\frac{\boldsymbol{U}}{\boldsymbol{gprot}}\right) = (\mathbf{2952.09} + \mathbf{163.41}) \div \mathbf{591.94} \div \mathbf{10} \times \mathbf{10} \div \mathbf{3.94}$$

$$= \mathbf{1.34}\ \boldsymbol{U/gprot}$$

## Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.



**Email:** info@assaygenie.com **Web:** www.assaygenie.com

## Notes:



**Email:** info@assaygenie.com **Web:** www.assaygenie.com

## Notes:



**Email:** info@assaygenie.com **Web:** www.assaygenie.com

**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.

## Contact Details

**Email:** info@assaygenie.com

**Web:** www.assayenie.com