TECHNICAL MANUAL

Total Superoxide Dismutase (T-SOD) Activity Assay Kit (Hydroxylamine)

- **SKU CODE:** MAES0338
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Operating steps
- Calculation
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can be used to measure total superoxide dismutase (T-SOD) activity in serum, plasma, urine, cells, cell culture supernatant, and tissue samples.

3. Detection principle

The superoxide anion free radical (O2•-) can be produced by the xanthine and xanthine oxidase reaction system. O2•- oxidizes hydroxylamine to form nitrite, which turns purple under the reaction with developer. When the measured samples contain SOD, the SOD can specifically inhibit superoxide anion free radicals (O2•-). The inhibitory effect of SOD reduces the formation of nitrite, resulting in a lower absorbance value in the sample tube compared to the control tube. The SOD content of the sample is calculated according to the computational formula.

4. Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 2	Nitrosogenic Agent	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 4	Enzyme Stock Solution	0.3 mL × 1 vial	0.6 mL × 1 vial	-20°C, 12 months
Reagent 5	Enzyme Diluent	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 6	Chromogenic Agent A	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, protected from light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, protected from light
Reagent 8	Chromogenic Agent C	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (550 nm), Micropipettor, Vortex mixer, Water bath, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

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 small volume reagents, please centrifuge before use to obtain sufficient amounts of reagents.

6. Reagent preparation

1. Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of buffer working solution:** For each tube, prepare 1.0 mL of buffer working solution by mixing 100 µL of buffer solution with 900 µL of double distilled water. Mix well. Store at 2-8°C for 3 months.
3. **Preparation of enzyme stock working solution:** For each tube, prepare 100 µL of enzyme stock working solution by mixing 5 µL of enzyme stock solution with 95 µL of enzyme diluent. Mix well. Store at 2-8°C for 3 days.
4. **Preparation of chromogenic agent A application solution:** Dissolve one vial of chromogenic agent A with 70-80°C double distilled water to a final volume of 90 mL. Store at 2-8°C for 3 months protected from light.
5. **Preparation of chromogenic agent B application solution:** Dissolve one vial of chromogenic agent B with double distilled water to a final volume of 90 mL. Store at 2-8°C for 1 month protected from light.

6. **Preparation of chromogenic agent:** For each tube, prepare 2.0 mL of chromogenic agent by mixing 750 µL of chromogenic agent A application solution, 750 µL of chromogenic agent B application solution, and 500 µL of chromogenic agent C. Mix well. The chromogenic agent should be prepared fresh. Keep at 4°C protected from light during use.

7. Sample preparation

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 one month.

Urine: Collect fresh urine and centrifuge at 10,000 xg for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine 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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 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 (MAES0177).

Cells:

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

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 10^6 cells in 300-500 µL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 1,500 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 (MAES0177).

Dilution of sample:

The inhibition ratio of this kit is 15-55%, with an optimal inhibition ratio of 25-45%. When the inhibition ratio is 30-40%, the corresponding sampling volume is the optimal sampling volume.

Inhibition ratio = $(OD_{control} - OD_{sample}) / OD_{control} \times 100\%$

If inhibition ratio > 50%, dilute the sample or decrease the sampling volume. If inhibition ratio < 15%, increase the sampling volume.

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

HepG2 supernatant: 1 (50 µL)

HepG2 cell: 8-10 (25 µL)

Mouse serum: 3-5 (20 µL)

10% Mouse liver tissue homogenate: 40-60 (20 µL)

10% Rat kidney tissue homogenate: 15-20 (20 µL)

Human urine: 1 (25 µL)

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The incubation time is 40 min. The incubation time can be extended to 45 min when the room temperature is lower than 20°C. The incubation temperature (37°C) should be stable.
2. It is recommended to use heparin as anticoagulant instead of EDTA.
3. The inhibition ratio of this kit is 15-55%, with an optimal inhibition ratio of 25-45%. When the inhibition ratio is 30-40%, the corresponding sampling volume is the optimal sampling volume. Inhibition ratio = (OD_control - OD_sample) / OD_control × 100%. If inhibition ratio > 50%, dilute the sample or decrease the sampling volume. If inhibition ratio < 15%, increase the sampling volume.

9. Operating steps

1. Sample tube: Add 1 mL of buffer working solution and a* mL sample to the sample tubes. Control tube: Add 1 mL of buffer working solution and a* mL double distilled water to the control tubes.
2. Add 0.1 mL of nitrosogenic agent, 0.1 mL of substrate solution, and 0.1 mL of enzyme stock working solution successively into each tube.
3. Mix fully with a vortex mixer and incubate at 37°C for 40 min.
4. Add 2 mL of chromogenic agent into each tube.
5. Mix fully and stand for 10 min at room temperature.
6. Set to zero with double distilled water and measure the OD value of each tube at 550 nm with a 1 cm optical path quartz cuvette.

10. Calculation

Serum (plasma) samples, culture cell supernatant, and other liquid samples:

Definition: The amount of SOD when the inhibition ratio reaches 50% in 1 mL reaction solution is defined as 1 SOD activity unit (U).

T-SOD activity (U/mL) = i ÷ 50% × V_1/V_2 × f

2. Tissue samples and cell samples:

Definition: The amount of SOD when the inhibition ratio reaches 50% of 1 mg tissue protein in 1 mL reaction solution is defined as 1 SOD activity unit (U).

T-SOD activity (U/mgprot) = i ÷ 50% × V_1/V_2 × f ÷ C_{pr}

Where:

i: inhibition ratio, Inhibition ratio = ($OD_{control}$ - OD_{sample}) / $OD_{control}$ × 100%

V_1: total volume of reaction solution, mL

V_2: volume of sample added, mL

f: dilution factor of sample before test

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

Note: If the optimal sampling volume (a*) is the same, only one control tube needs to be assayed.

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/mL)	15.00	78.00	124.00
%CV	3.2	2.7	2.5

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/mL)	15.00	78.00	124.00
%CV	5.9	6.7	6.3

Recovery

Three samples of high, middle, and low concentration were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 105%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	26	106	135
Observed Conc. (U/mL)	28.1	110.2	139.1
Recovery rate (%)	108	104	103

Sensitivity

The analytical sensitivity of the assay is 4.7 U/mL T-SOD. 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.

12. Appendix II Example Analysis

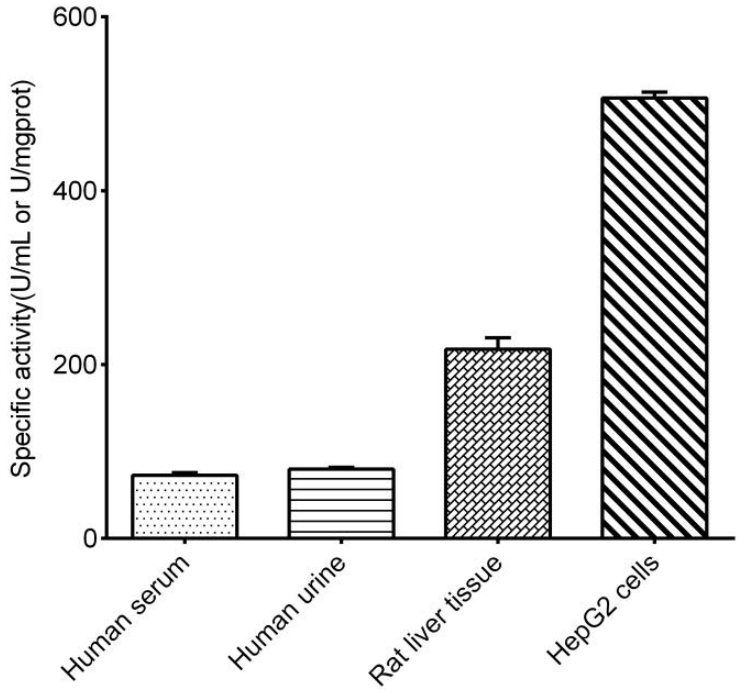
Example analysis:

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) 10-fold before use. Take 10 µL sample dilution and carry out the assay according to the operation steps. The results are as follows:

The average OD value of the control tube is 0.343, the average OD value of the sample tube is 0.212, the concentration of protein in the sample is 11.61 mgprot/mL, and the calculation result is:

T-SOD activity (U/mgprot) = (0.343-0.212)/0.343 ÷ 50% × 3.31/0.01 × 10 ÷ 11.61 = 217.77 U/mgprot

Detect human serum (V_2=30 μL), human urine (V_2=30 μL), 10% rat liver tissue homogenate (the concentration of protein is 11.61 mgprot/mL, diluted 10-fold, V_2=10 μL), HepG2 cells (the concentration of protein is 3.18 mgprot/mL, diluted 4-fold, V_2=5 μL) according to the protocol. The result is 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.