TECHNICAL MANUAL

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

- **SKU CODE:** MAES0032
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Add samples and reagents to wells
- Incubate and develop color
- Measure absorbance

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

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500Assays)	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8°C, 12 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8°C, 12 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8°C, 12 months
Reagent 4	Enzyme Stock Solution	0.03 mL × 1 vial	0.06 mL × 1 vial	0.06 mL × 5 vials	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500Assays)	Storage
Reagent 5	Enzyme Diluent	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8°C, 12 months
Reagent 6	Chromogenic Agent A	Powder ×1 vial	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months, protect from light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months, protect from light
Reagent 8	Chromogenic Agent C	6 mL × 1 vial	6 mL × 1 vial	6 mL × 5 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. Materials prepared by users

Instruments:

Microplate reader (520-550 nm, optimum wavelength: 530 nm), micropipettor, centrifuge, incubator

Reagents:

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

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 well, prepare 90 µL of buffer working solution (mix well 9 µL of buffer solution and 81 µL of double distilled water). Store at 2-8°C for 3 months.
3. **Preparation of enzyme stock working solution:** For each well, prepare 40 µL of enzyme stock working solution (mix well 2 µL of enzyme stock solution and 38 µL of enzyme diluent). 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 9 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 9 mL. Store at 2-8°C for 1 month protected from light.
6. **Preparation of chromogenic agent:** For each well, prepare 180 µL of chromogenic agent (mix well 67.5 µL of chromogenic agent A application solution, 67.5 µL of chromogenic agent B application solution and 45 µL of chromogenic agent C). The chromogenic agent should be prepared fresh. Keep it at 4°C protected from light during use.
7. **Preparation of enzyme working solution:** For each well, prepare 30 µL of enzyme working solution (mix well 10 µL of nitrosogenic agent, 10 µL of substrate solution and 10 µL of enzyme stock working solution). The enzyme working solution should be prepared fresh, and it must be used within 20 minutes.
8. **Preparation of non-enzyme working solution:** For each well, prepare 30 µL of non-enzyme working solution (mix well 10 µL of nitrosogenic agent, 10 µL of substrate solution and 10 µL of enzyme diluent). Store at 2-8°C for 1 month protected from light. The non-enzyme working solution should be prepared fresh, and it must be used within 20 minutes. (Only need to prepare the solution for 3 wells)

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not tested on the same day, serum or plasma can be stored at -80°C for one month.

Urine: Collect fresh urine and centrifuge at 10,000 ×g for 15 minutes to remove insoluble material. Collect supernatant and keep on ice for detection. If not tested on the same day, 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 ×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

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 ×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

Dilution of sample:

Determine the optimum dilution factor of the sample before the formal experiment. Calculate the inhibition ratio of serial dilution multiples of sample, and choose the optimum dilution factor when the inhibition ratio is in the range of 25%-45%.

Inhibition ratio = ($A_{control}$ - $A_{control}$ blank - (A_{sample} - $A_{control}$ blank)) / ($A_{control}$ - $A_{control}$ blank) × 100%

= ($A_{control}$ - A_{sample}) / ($A_{control}$ - $A_{control}$ blank) × 100%

The recommended dilution factors for different samples are as follows (for reference only):

Human serum: 2-4 fold

Rat serum: 4-6 fold

Mouse serum: 4-6 fold

HepG2 cells: 15-30 fold

Human urine: 2-5 fold

10% Mouse liver tissue homogenate: 160-200 fold

10% Epipremnum aureum tissue homogenate: 3-5 fold

10% Mouse brain tissue homogenate: 80-100 fold

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. Determine the optimum dilution factor of the sample before the formal experiment. Calculate the inhibition ratio of serial dilution multiples of sample, and choose the optimum dilution factor when the inhibition ratio is in the range of 25%-45%. Inhibition ratio = (A_control - A_control blank - (A_sample - A_control blank)) / (A_control - A_control blank) × 100% = (A_control - A_sample) / (A_control - A_control blank) × 100%. If inhibition ratio is more than 50%, dilute the sample

and then carry out the assay. If inhibition ratio is less than 10%, increase the dilution multiple.

2. EDTA should not be used as an anticoagulant; heparin plasma is recommended.

3. The prepared enzyme working solution must be used within 20 minutes.

9. Operating steps

1. Control blank well: Add 5 µL of PBS (0.01 M, pH 7.4) into the control blank wells. Sample well: Add 5 µL of sample into the sample wells. Control well: Add 5 µL of PBS (0.01 M, pH 7.4) into the control wells.

2. Add 90 µL of buffer working solution into each well.

3. Add 30 µL of enzyme working solution to the control wells and sample wells. Add 30 µL of non-enzyme working solution to the control blank wells.

4. Mix thoroughly for 10 seconds with microplate reader and cover the plate with sealer. Incubate at 37°C for 50 minutes.

5. Add 180 µL of chromogenic agent to each well.

6. Mix thoroughly for 10 seconds with microplate reader and stand for 10 minutes at room temperature. Measure the OD values of each well at 550 nm with microplate reader.

10. Calculation

The sample:

1. Serum (plasma) samples, cultured cells 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/mg_{prot}) = i ÷ 50% × V_1/V_2 × f ÷ C_{pr}

[Note]

i: Inhibition ratio of SOD (%), i = $(A_1-A_3-(A_2-A_3))/(A_1-A_3)$ × 100% = $(A_1-A_2)/(A_1-A_3)$ × 100%

A_1: The OD value of control well at 550 nm.

A_2: The OD value of sample well at 550 nm.

A_3: The OD value of control blank well at 550 nm.

V_1: The total volume of reaction solution, mL.

V_2: The volume of sample added into the reaction system, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, g_{prot}/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/mL)	3.50	25.00	45.00
%CV	5.9	5.4	5.2

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)	3.50	25.00	45.00
%CV	5.5	5.3	6.0

Recovery

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

Recovery

Sample 1	Sample 2	Sample 3
15	34	55
16.1	36.0	56.1
107	106	102

Sensitivity

The analytical sensitivity of the assay is 2.4 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

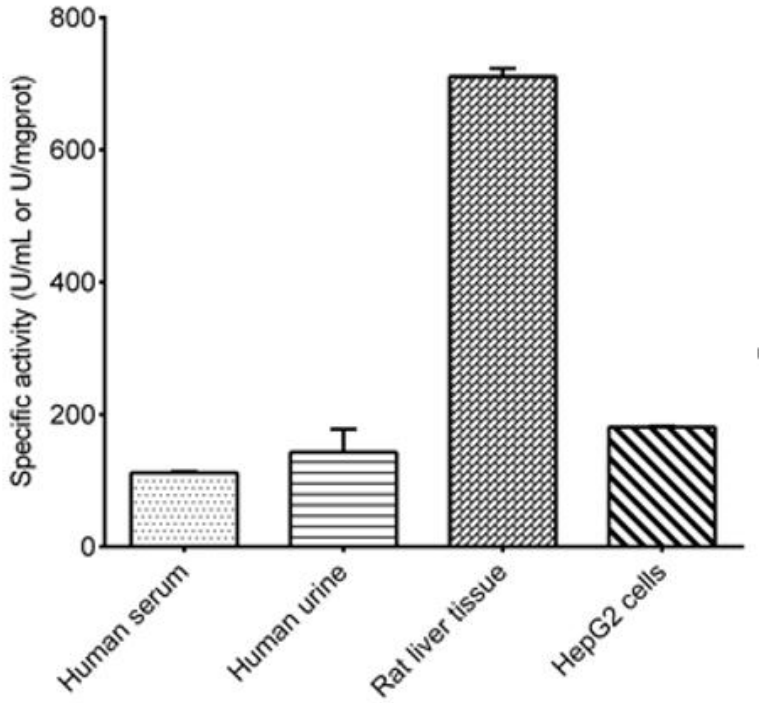
Example analysis:

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) 160 times before use, take 5 μL sample dilution, and carry out the assay according to the operation steps. The results are as follows:

The average OD value of the sample well is 0.248, the average OD value of the control well is 0.333, the average OD value of the control blank well is 0.132, the concentration of protein in sample is 11.61 mg_{prot}/mL, and the calculation result is:

T-SOD activity (U/mg_{prot}) = (0.333 - 0.248)/(0.333 - 0.132) ÷ 50% × 0.305/0.005 × 160 ÷ 11.61 = 711 U/mg_{prot}

Detect human serum (the concentration of protein in sample, dilute 2 times, V=5 μL), human urine (the concentration of protein in sample, dilute 3 times, V=5 μL), rat liver tissue homogenate (the concentration of protein is 11.61 mg_{prot}/mL, dilute 160 times, V=5 μL), HepG2 cells (the concentration of protein is 6.09 mg/mL, dilute 20 times, V=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.