TECHNICAL MANUAL

CuZn/Mn Superoxide Dismutase (CuZn-SOD/Mn-SOD) Activity Assay Kit (Hydroxylamine)

- **SKU CODE:** MAES0035
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample pretreatment
- Reagent preparation
- Add samples to microplate
- Add buffer working solution
- Add enzyme/non-enzyme working solution
- Incubate at 37°C for 50 min
- Add chromogenic agent
- Measure absorbance at 550 nm

2. Intended use

This kit can be used to measure T-SOD, CuZn-SOD, and Mn-SOD activity in serum, plasma, urine, cells, cell culture supernatant, and tissue homogenate samples.

3. Detection principle

Superoxide anion (O2•-) produced by the xanthine and xanthine oxidase system can oxidize hydroxylamine to form nitrite, which appears purplish red after chromogenic reaction. The SOD in the sample has a specific inhibitory effect on superoxide anion (O2•-) and can reduce the nitrite content. The OD value is lower than the control, and SOD activity is calculated through the formula.

There are two kinds of SOD (CuZn-SOD and Mn-SOD) in the cells of higher animals, and their sum equals the total SOD. The activity of Mn-SOD in the sample will be lost after sample pretreatment, but the activity of CuZn-SOD will not.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8°C, 12 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 4	Enzyme Stock Solution	0.03 mL × 1 vial	0.06 mL × 1 vial	-20°C, 12 months
Reagent 5	Enzyme Diluent	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8°C, 12 months
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	6 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months
Reagent 9	Extracting Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months, protected from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (530-570 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

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:** Before testing, prepare sufficient enzyme stock working solution according to the test wells. For example, prepare 100 µL of enzyme stock working solution (mix well 5 µL of enzyme stock solution and 95 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 200 µL of chromogenic agent (mix well 75 µL of chromogenic agent A application solution, 75 µL of chromogenic agent B application solution and 50 µL of chromogenic agent C). The chromogenic agent should be prepared on spot. Store at 2-8°C for 1 month protected from light.
7. **Preparation of enzyme working solution:** (Operate on ice) 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 on spot, and it must be used within 20 min.
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).

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 a month.

Urine: Collect fresh urine and centrifuge at 10,000×g 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 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 10,000×g 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 normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g 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 optimal sampling volumes are different for different species, and SOD levels also vary for different samples. It is recommended to take 2-3 samples to do a pre-experiment, diluting a series of dilutions and determining the dilution factor when the SOD inhibition ratio is 15%-55% (the optimal inhibition ratio is in the range of 25%-45%) before the formal experiment.

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

- Mouse serum: 5-10
- Rat serum: 6-15
- Urine: 2-3
- Human hydrothorax: 2-3
- 10% Mouse liver tissue homogenate: 100-200
- 10% Mouse brain tissue homogenate: 20-30
- 10% Mouse kidney tissue homogenate: 50-120
- 10% Rat kidney tissue homogenate: 50-120
- HepG2 cells (5.21 mgprot/mL): 15-25
- Cell culture supernatant: 1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. The key points of the assay

1. Determine optimal sampling volume of each sample before formal experiment. Calculate the inhibition ratio of serial sampling volumes, and choose the optimal sampling volume when inhibition ratio is in the range of 25%-45%.

2. The optimal sampling volumes are different for different species, and SOD levels also vary for different samples. So it is best to do a pre-test to determine optimal sampling volume for a new sample.
3. It is best to reserve 3 paralleled tubes with different sampling volumes in pre-test for determining the optimal sampling volume.
4. Adjust sampling volume: If inhibition ratio >55%, need to dilute the sample or decrease the sampling volume before testing. If inhibition ratio <15%, need to increase the sampling volume.
5. There should be no bubbles in the wells of the microplate when measuring the OD value.
6. A multichannel pipettor is recommended to shorten the time and reduce the error between wells.

9. Operating steps

Sample pretreatment:

1. Take 0.1 mL sample and add 0.1 mL extracting solution. Mix thoroughly with a vortex mixer for 1 min and centrifuge at 3,500×g for 15 min. Take supernatant for CuZn-SOD measurement.

2. Take 0.1 mL normal saline and add 0.1 mL extracting solution. Mix thoroughly for 1 min with a vortex mixer and centrifuge at 3,500×g for 15 min. Take supernatant as control of CuZn-SOD.

The measurement of samples:

1. Blank well of T-SOD: add 5 μL of double distilled water into the corresponding wells.

Control well of T-SOD: add 5 μL of double distilled water to the corresponding wells.

Sample well of T-SOD: add 5 μL of sample to the corresponding wells.

Blank well of CuZn-SOD: add 5 μL of supernatant as control of CuZn-SOD into the corresponding wells.

Control well of CuZn-SOD: add 5 μL of supernatant as control of CuZn-SOD to the corresponding wells.

Sample well of CuZn-SOD: add 5 μL of supernatant for CuZn-SOD to the corresponding wells.

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

3. Add 30 μL of enzyme working solution to control well of T-SOD, sample well of T-SOD, control well of CuZn-SOD, and sample well of CuZn-SOD.

Add 30 µL of non-enzyme working solution to blank well of T-SOD and blank well of CuZn-SOD.

4. Mix fully for 10 s with microplate reader and incubate at 37°C for 50 min.

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

6. Mix fully for 10 s with microplate reader and stand for 10 min at room temperature. Measure the OD values of each well at 550 nm with microplate reader.

10. Calculation

The sample:

1. Serum (plasma), cell culture medium and other liquid samples:

Definition: When SOD inhibition ratio in 1 mL reaction system reaches 50%, the corresponding enzyme level is 1 SOD activity unit (U).

T-SOD activity (U/mL) = i1 ÷ 50% × V_1/V_2 × f

CuZn-SOD activity (U/mL) = i2 ÷ 50% × V_1/V_2 × f

2. Tissue and cell samples:

Definition: When SOD inhibition ratio of 1 mg of tissue protein in 1 mL reaction system reaches 50%, the corresponding enzyme level is 1 SOD activity unit (U).

T-SOD activity (U/mL) = i1 ÷ 50% × V_1/V_2 × f ÷ Cpr

CuZn-SOD activity (U/mL) = i2 ÷ 50% × V_1/V_2 × f ÷ Cpr

Mn-SOD activity = T-SOD activity - CuZn-SOD activity

Note:

i1: Inhibition ratio of T-SOD.

i1 (%) = $(A_1-A_3-(A_2-A_3))/(A_1-A_3) \times 100\% = (A_1-A_2)/(A_1-A_3) \times 100\%$

i2: Inhibition ratio of CuZn-SOD.

i2 (%) = $(A_4-A_6-(A_5-A_6))/(A_4-A_6) \times 100\% = (A_4-A_5)/(A_4-A_6) \times 100\%$

A_1: The OD value of $\text{T-SOD}_{\text{Control}}$.

A_2: The OD value of $\text{T-SOD}_{\text{Sample}}$.

A_3: The OD value of $\text{T-SOD}_{\text{Blank}}$.

A_4: The OD value of $\text{CuZn-SOD}_{\text{Control}}$.

A_5: The OD value of $\text{CuZn-SOD}_{\text{Sample}}$.

A_6: The OD value of $\text{CuZn-SOD}_{\text{Blank}}$.

V_1: The total volume of the reaction system (mL).

V_2: The volume of sample added to the reaction system (mL).

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample, gprot/L.

11. Appendix

Performance Characteristics

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)	2.60	29.50	48.20
%CV	5.6	4.9	4.8

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)	2.60	29.50	48.20
%CV	10.2	9.2	9.4

Recovery

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

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	13	34	55
Observed Conc. (U/mL)	13.1	33.3	53.9
Recovery rate (%)	101	98	98

Sensitivity

The analytical sensitivity of the assay is 1.35 U/mL. 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.

Example Analysis

The detection of T-SOD: Take 10% mouse liver tissue homogenate, dilute 150 times with normal saline (0.9% NaCl), then take 5 μL of diluted sample, and carry out the assay according to the operation steps. The results are as follows:

The average OD value of $T\text{-}SOD_{Sample}$ is 0.344, the average OD value of $T\text{-}SOD_{Control}$ is 0.546, the average OD value of $T\text{-}SOD_{Blank}$ is 0.121, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

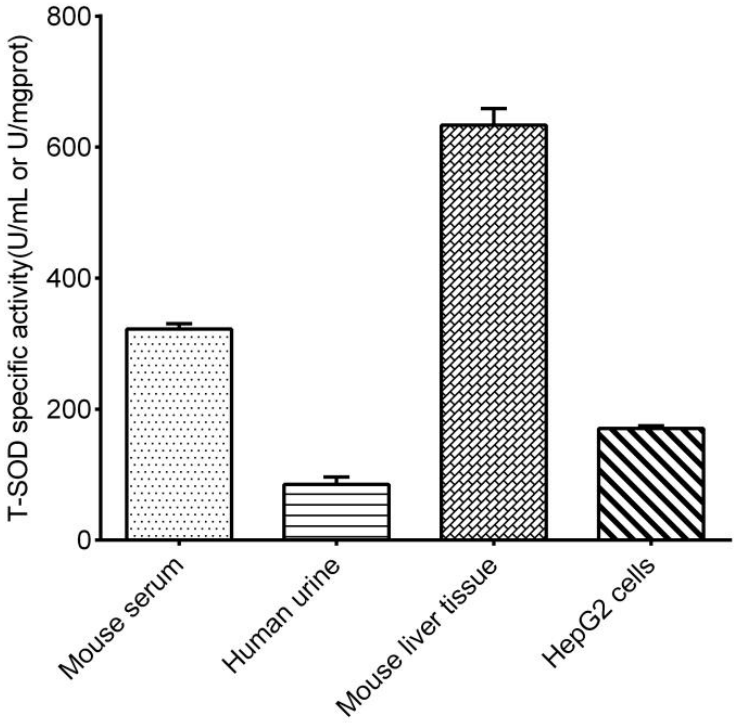
T-SOD activity (U/mgprot) = (0.546-0.344)/(0.546-0.121) ÷ 50% × 0.305/0.005 × 150 ÷ 13.72 = 633.96 U/mgprot

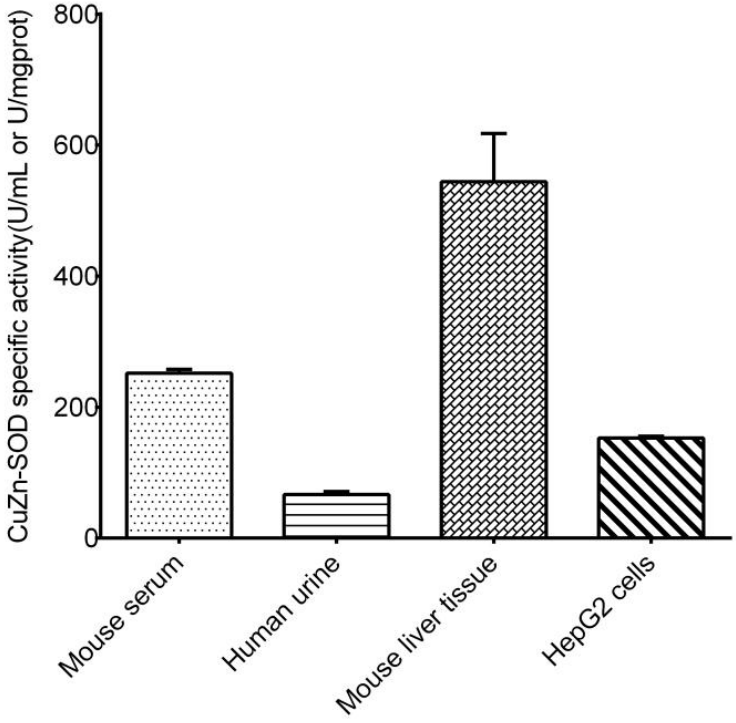
The detection of CuZn-SOD: pretreat the diluted sample with extracting solution, and carry out the assay according to the operation steps. The results are as follows:

The average OD value of $CuZn\text{-}SOD_{Sample}$ is 0.400, the average OD value of $CuZn\text{-}SOD_{Control}$ is 0.590, the average OD value of $CuZn\text{-}SOD_{Blank}$ is 0.124, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

CuZn-SOD activity (U/mgprot) = (0.590-0.400)/(0.590-0.124) ÷ 50% × 0.305/0.005 × 150 ÷ 13.72 = 543.83 U/mgprot

Detect mouse serum (diluted 5 times), human urine (diluted 2 times), 10% mouse liver tissue homogenate (the concentration of protein is 13.72 mgprot/mL, diluted 150 times) and HepG2 cells (the concentration of protein is 5.21 mgprot/mL, diluted 20 times) according to the protocol. The results are as follows:





12. Statement

1. This assay kit is for Research Use Only. We will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be performed on the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie will guarantee the quality of the kits only and is NOT responsible for sample consumption caused by using the assay kits. It is advisable to calculate the possible usage of samples and reserve sufficient samples before use.

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.