TECHNICAL MANUAL

Total Superoxide Dismutase (T-SOD) Activity Assay Kit (WST-1)

- **SKU CODE:** MAES0034
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples to wells
- Add substrate application solution
- Incubate and measure

2. Intended use

This kit can be used to measure total superoxide dismutase (T-SOD) activity in serum, plasma, pleural effusion, ascites, urine, cells, and various animal and plant tissue samples.

3. Detection principle

The assay is based on the inhibition of WST-1 reduction by SOD. Superoxide anions generated in the reaction system reduce WST-1 to a water-soluble formazan dye. SOD activity negatively correlates with the formazan dye formation, which is quantified colorimetrically at 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Buffer Solution	12 mL x 1 vial	24 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months
Reagent 2	Substrate Solution	0.07 mL x 1 vial	0.14 mL x 1 vial	1 mL x 1 vial	2-8°C, 12 months, protect from light
Reagent 3	Enzyme Stock Solution	0.15 mL x 1 vial	0.3 mL x 1 vial	1.5 mL x 1 vial	-20°C, 12 months
Reagent 4	Enzyme Diluent	1.5 mL x 1 vial	1.5 mL x 2 vials	15 mL x 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500 Assays)	Storage
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (440-460 nm), Micropipettor, Multichannel pipettor, Vortex mixer, 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. Allow other reagents to equilibrate to room temperature before use.
2. **Preparation of substrate application solution:** Before testing, prepare sufficient substrate application solution according to the number of test wells. For example, to prepare 1005 µL of substrate application solution, thoroughly mix 1000 µL of buffer solution with 5 µL of substrate solution. Store at 2-8°C for 7 days.
3. **Preparation of enzyme working solution:** Before testing, prepare sufficient enzyme working solution according to the number of test wells. For example, to prepare 220 µL of enzyme working solution, thoroughly mix 20 µL of enzyme stock solution with 200 µL of enzyme diluent (Note: Please perform this step on ice). Store at 2-8°C for 3 days.

7. Sample preparation

Sample requirements: The samples should not contain SDS, Tween20, NP-40, Triton X-100 or other detergents, and should not contain DTT, 2-mercaptoethanol or other reducing agents.

Sample preparation:

Serum and plasma: Detect directly. If not analyzed 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 x g for 15 min to remove insoluble material. Collect supernatant and keep on ice for detection. If not analyzed 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 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep 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 10,000 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Sample dilution:

The optimal sampling volume varies for different species, and SOD levels differ among sample types. It is recommended to test 2-3 samples in a preliminary experiment, preparing a series of dilutions to determine the dilution factor when the SOD inhibition ratio is 25%-65% before the formal experiment.

Recommended dilution factors for different samples (for reference only):

Human serum: 3-5

Rat serum: 20-30

Urine: 1

Human pleural effusion: 2

Cell culture supernatant: 2-3

10% Rat liver tissue homogenate: 340-370

10% Rat heart tissue homogenate: 80-100

10% Rat kidney tissue homogenate: 100-120

10% Rat brain tissue homogenate: 50-100

HepG2 cells homogenate (3 mg protein/mL): 30-40

10% Plant tissue homogenate: 5-10

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

8. The key points of the assay

1. The inhibition ratio of SOD should be 25%-65%.

2. Prevent bubble formation when adding liquid to the microplate.

3. Superoxide is formed immediately after substrate application solution is added. A multichannel pipettor is recommended to shorten the time and reduce error between wells.

9. Operating steps

1. Well setup: Control well: Add 20 µL of double distilled water and 20 µL of enzyme working solution into the control wells. Blank Control well: Add 20 µL of double distilled water and 20 µL of enzyme diluent into the Blank Control wells. Sample well: Add 20 µL of sample and 20 µL of enzyme working solution into the sample wells.

2. Add 200 µL of substrate application solution with a multichannel pipettor into each well and mix thoroughly.

3. Incubate at 37°C for 20 min. Measure the OD values of each well at 450 nm with a microplate reader.

10. Calculation

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

$i = (A_{Control} - A_{BlankControl}) - (A_{Sample} - A_{BlankControl}) / (A_{Control} - A_{BlankControl}) \times 100\%$

For samples:

1. Serum (plasma) sample:

SOD activity (U/mL) = $i \div 50\% \times V_1/V_2 \times f$

2. Tissue sample and cell sample:

SOD activity (U/mg protein) = $i \div 50\% \times V_1/V_2 \times f \div C_{pr}$

Where:

i: Inhibition ratio of SOD (%)

V_1: The total volume of reaction, 240 µL

V_2: The volume of sample added to the reaction, 20 µL

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, g protein/L

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)	1.20	8.40	12.50
%CV	3.2	3.0	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)	1.20	8.40	12.50
%CV	3.4	3.8	3.9

Recovery

Three samples of high, medium, and low concentrations were tested in parallel six times each to obtain an average recovery rate of 98%.

Recovery

Parameters	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	3.5	9.6	13
Observed Conc. (U/mL)	3.5	9.1	12.6
Recovery rate (%)	99	95	97

Sensitivity

The analytical sensitivity of the assay is 0.2 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.

11. Appendix II Example Analysis

Example analysis:

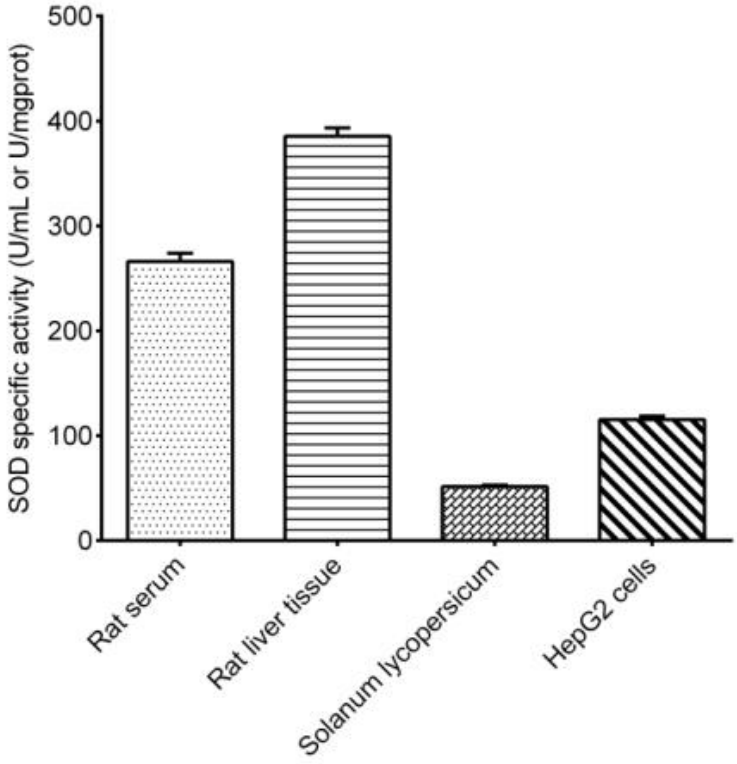
Human serum was diluted 5-fold with PBS, then 0.02 mL of diluted sample was taken and assayed according to the operating steps. The results were as follows:

The average OD value of the control was 0.608, the average OD value of Blank Control was 0.048, the average OD value of the sample was 0.388, and the calculation result was:

Inhibition ratio of SOD (%) = [(0.608-0.048) - (0.388-0.048)]/(0.608-0.048) × 100% = 39.29%

SOD activity (U/mL) = 39.29% ÷ 50% × 0.24/0.02 × 5 = 47.15 (U/mL)

Rat serum (diluted 20-fold), 10% rat liver tissue homogenate (protein concentration: 10.67 mg protein/mL, diluted 360-fold), 20% tomato tissue homogenate (protein concentration: 2.44 mg protein/mL, diluted 10-fold), and HepG2 cells (protein concentration: 3.18 mg protein/mL, diluted 30-fold) were analyzed according to the protocol, with the following results:



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 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.