TECHNICAL MANUAL

Inhibition And Production Of Superoxide Anionic Colorimetric Assay Kit (WST-1)

- **SKU CODE:** MAES0014
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add reagents to wells
- Incubate at 37°C for 20 min
- Measure OD values at 450 nm

2. Intended use

This kit can be used to measure the activity of inhibition of superoxide anion radical in serum, plasma, urine, cells and cellular supernatant, or the activity of production of superoxide anion radical in leukocyte samples.

3. Detection principle

Superoxide anion free radicals are produced through the reaction system of xanthine and xanthine oxidase. WST-1 (a water-soluble tetrazolium salt) can react with the generated superoxide anion to produce water-soluble formazan. When the tested sample contains superoxide anion free radical inhibitor, it can inhibit the formation of formazan. When the tested sample contains substances that produce superoxide anion free radical, it can promote the formation of formazan dye. By colorimetric analysis of WST-1 products, the units of activity of inhibition or production of superoxide anion radical in samples can be calculated.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate Solution	0.07 mL × 1 vial	0.14 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Enzyme Stock Solution	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months
Reagent 4	Enzyme Diluent	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 5	VC Standard	Powder × 3 vials	Powder × 3 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (440-460 nm), Micropipettor, Multi-channel pipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

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

6. Reagent preparation

1. Keep enzyme stock solution on ice for use. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Before testing, prepare sufficient enzyme working solution according to the number of test wells. For example, prepare 55 µL of enzyme working solution by mixing well 5 µL of enzyme stock solution and 50 µL of enzyme diluent. Store at 2-8°C for 3 days. (Enzyme stock solution should melt slowly on ice. It is recommended to aliquot the enzyme stock solution into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.)
3. **Preparation of substrate application solution:** Before testing, prepare sufficient substrate application solution according to the number of test wells. For example, prepare 1005 µL of substrate application solution by mixing well 5 µL of substrate solution and 1000 µL of buffer solution. Store at 2-8°C for 7 days.
4. **Preparation of 5 mg/mL standard solution:** Dissolve one vial of VC standard with 1 mL of double distilled water, mix well.
5. **Preparation of 0.05 mg/mL standard solution:** Before testing, prepare sufficient 0.05 mg/mL standard solution according to the number of test wells. For example, prepare 100 µL of 0.05 mg/mL standard solution by mixing well 1 µL of 5 mg/mL

standard solution and 99 µL of double distilled water. VC standard is easily oxidized; it is best to use within 30 minutes.

7. Sample preparation

Sample preparation requirements:

Samples should not contain decontamination agents such as SDS, Tween-20, NP-40, Triton X-100, nor reductive reagents such as DTT, 2-mercaptoethanol.

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.

Saliva: Rinse mouth with clear water, collect saliva 30 minutes later, centrifuge at 10,000×g for 10 minutes at 4°C. Take the supernatant and preserve it on ice for detection.

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 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Cell (adherent or suspension) samples:

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

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

3. Homogenize 1×10^6 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×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Inhibition ratio of sample:

Before the formal experiment, choose 2-3 samples for serial dilution and determine the dilution factor when the SOD inhibition ratio is 30%-65% (the optimal inhibition ratio is 40%-60%).

Inhibition ratio = $[(A_1 - A_2) - (A_5 - A_6)] / (A_1 - A_2) \times 100\%$

Adjust sampling volume: If inhibition ratio > 65%, dilute the sample or decrease the sampling volume. If inhibition ratio < 30%, increase the sampling volume.

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

Human serum: 4-7

Mouse serum: 15-25

Rat serum: 25-35

Human saliva: 1

HepG2 culture supernatant: 1

10% Rat brain tissue homogenate: 150-200

10% Rat liver tissue homogenate: 500-600

10% Mouse liver tissue homogenate: 500-600

10% Mouse heart tissue homogenate: 150-200

10% Epipremnum aureum tissue homogenate: 20-30

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

8. The key points of the assay

1. In order to reduce errors between different wells, the multi-channel pipettor is recommended.
2. VC standard is easily oxidized; it is best to use the standard solution within 30 minutes.
3. Prevent the formation of bubbles when transferring supernatant into the microplate.
4. Samples should not contain decontamination agents such as SDS, Tween-20, NP-40, Triton X-100, nor reductive reagents such as DTT, 2-mercaptoethanol.
5. Before the formal experiment, choose one or two samples for serial dilution and determine the dilution factor when the SOD inhibition ratio is 30%-65% (the optimal inhibition ratio is 40%-60%).

9. Operating steps

1. Add reagents to wells: Control well: add 20 μL of double distilled water and 20 μL of enzyme working solution to the corresponding wells. Blank control well: add 20 μL of double distilled water and 20 μL of enzyme diluent to the corresponding wells. Standard well: add 20 μL of 0.05 mg/mL standard solution and 20 μL of

enzyme working solution to the corresponding wells. Blank standard well: add 20 µL of 0.05 mg/mL standard solution and 20 µL of enzyme diluent to the corresponding wells. Sample well: add 20 µL of sample and 20 µL of enzyme working solution to the corresponding wells. Blank sample well: add 20 µL of sample and 20 µL of enzyme diluent to the corresponding wells.

2. Add 200 µL of substrate application solution with a multi-channel pipettor into each well and mix thoroughly.
3. Incubate at 37°C for 20 minutes. Measure the OD values of each well with microplate reader at 450 nm.

10. Calculation

Calculation formula for the activity of inhibition of superoxide anion radical in serum, plasma, cellular supernatant:

Definition: In the reaction system, the amount of superoxide anion radical inhibited by 1 L of sample in 20 minutes at 37°C that is equivalent to inhibition by 1 mg of VC is defined as 1 unit.

The inhibition of superoxide anion radical (U/L) = $[(A_1 - A_2) - (A_5 - A_6)] / [(A_1 - A_2) - (A_3 - A_4)] \times C \times 1000 \times f$

2. Calculation formula for the activity of inhibition of superoxide anion radical in tissue and cells:

Definition: In the reaction system, the amount of superoxide anion radical inhibited by 1 g of sample in 20 minutes at 37°C that is equivalent to inhibition by 1 mg of VC is defined as 1 unit.

The inhibition of superoxide anion radical (U/gprot) = $[(A_1 - A_2) - (A_5 - A_6)] / [(A_1 - A_2) - (A_3 - A_4)] \times C \times 1000 \div Cpr \times f$

3. Calculation formula for the activity of production of superoxide anion radical:

(1) Liquid sample:

Definition: In the reaction system, the amount of superoxide anion radical produced by 1 L of substance in 20 minutes at 37°C that is equivalent to inhibition by 1 mg of VC is defined as 1 unit.

The production of superoxide anion radical (U/L) = $[(A_5 - A_6) - (A_1 - A_2)] / [(A_1 - A_2) - (A_3 - A_4)] \times C \times 1000 \times f$

(2) For solid samples:

Definition: In the reaction system, the amount of superoxide anion radical produced by 1 g of substance in 20 minutes at 37°C that is equivalent to inhibition by 1 mg of VC is defined as 1 unit.

The production of superoxide anion radical (U/gprot) = $[(A_5 - A_6) - (A_1 - A_2)] / [(A_1 - A_2) - (A_3 - A_4)] \times C \div C1 \times f$

Note:

A_1: The OD value of control

A_2: The OD value of blank control

A_3: The OD value of standard

A_4: The OD value of blank standard

A_5: The OD value of sample

A_6: The OD value of blank sample

C: The concentration of standard, 0.05 mg/mL

1000: Unit conversion, 1 L = 1000 mL

Cpr: The concentration of protein in sample, gprot/L

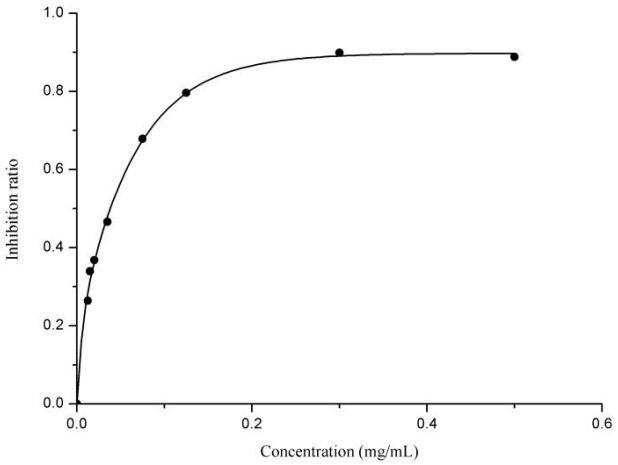
f: The dilution factor of sample before test

C1: The concentration of sample, g/L

11. Appendix I Performance Characteristics

Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mg/mL)	**0**	**0.0125**	**0.015**	**0.035**	**0.075**	**0.125**	**0.3**	**0.5**
OD of standard	0.567	0.411	0.386	0.311	0.218	0.167	0.202	0.31
	0.541	0.426	0.376	0.324	0.214	0.172	0.197	0.308
OD of blank standard	0.039	0.039	0.041	0.042	0.052	0.064	0.15	0.252
	0.039	0.04	0.041	0.043	0.049	0.065	0.145	0.251
Average OD of standard	0.554	0.419	0.381	0.318	0.216	0.17	0.2	0.309
Average OD of blank standard	0.039	0.04	0.041	0.043	0.051	0.065	0.148	0.252
Absolute OD	0.515	0.379	0.34	0.275	0.166	0.105	0.052	0.058
Inhibition ratio	0	26%	34%	47%	68%	80%	90%	89%

12. Appendix II Example Analysis

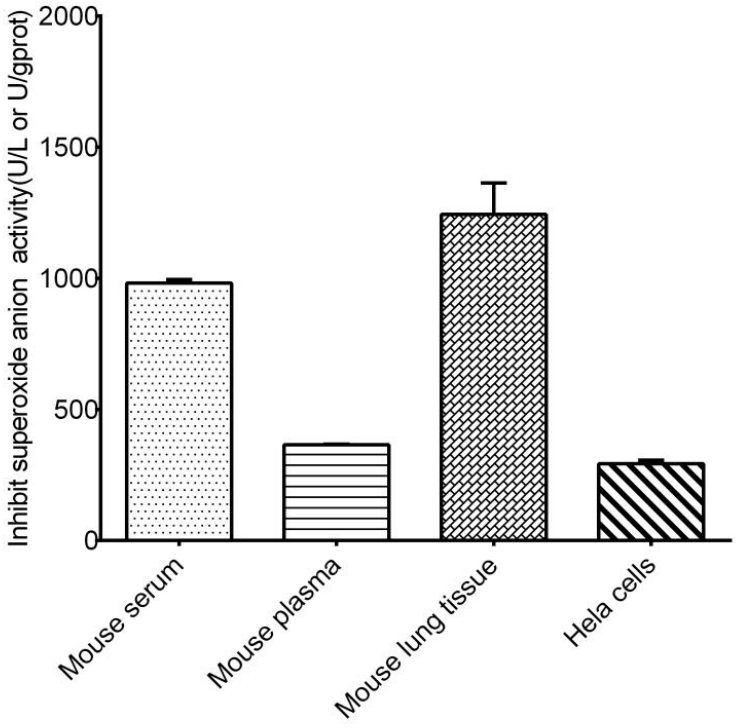
Example analysis:

Dilute 10% mouse lung tissue homogenate with normal saline (0.9% NaCl) 200-fold, take 20 μL of diluted sample and perform the assay according to the operating steps. The results are as follows:

The average OD value of control well is 0.588, the average OD value of blank control well is 0.045, the average OD value of standard well is 0.313, the average OD value of blank standard well is 0.043, the average OD value of sample well is 0.344, the average OD value of blank sample well is 0.040, the concentration of protein in 10% mouse lung tissue homogenate is 7.04 gprot/L, and the calculation result is:

The production of superoxide anion radical (U/gprot) = {(0.588-0.045) - (0.344-0.040)} ÷ {(0.588-0.045) - (0.313-0.043)} × 0.05 × 1000 × 200 ÷ 7.04 = 1243.5 U/gprot

Detect mouse serum (diluted 20-fold), mouse plasma (diluted 8-fold), 10% mouse lung tissue homogenate (the concentration of protein is 7.04 gprot/L, diluted 200-fold) and HeLa cells (the concentration of protein is 5.59 gprot/L, diluted 30-fold) 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 using the kit for clinical diagnosis or other purposes.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Please follow the instructions strictly during the experiments.
3. Protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range exactly, additional dilution or concentration should be performed on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operations, environmental conditions, and other factors. Assay Genie guarantees 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.