TECHNICAL MANUAL

Total Oxidant Status (TOS) Colorimetric Assay Kit

- **SKU CODE:** MAES0199
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add chromogenic agent
- Measure initial absorbance (A1)
- Add substrate and incubate
- Measure final absorbance (A2)
- Calculate results

2. Intended use

This kit is used for the determination of Total Oxidant Status (TOS) in cells, tissue, serum and other liquid samples.

3. Detection principle

Under acidic conditions, oxidizing materials in the sample can oxidize Fe2+ to Fe3+, which binds strongly with xylenol orange to produce a blue-purple complex. In acidic media, the reaction product shows an absorbance peak near 590 nm. Under fixed experimental conditions (e.g., defined concentration range and reaction time), the absorbance correlates linearly with the oxidant concentration, enabling indirect quantification of the sample's total oxidizing capacity.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500Assays)	Storage
Reagent 1	Chromogenic Agent	12 mL×1 vial	24 mL×1 vial	60 mL×2 vials	2-8 °C, 12 months, protect from light

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500Assays)	Storage
Reagent 2	Substrate	3 mL×1 vial	6 mL×1 vial	30 mL×1 vial	2-8 °C, 12 months, protect from light
Reagent 3	200 μmol/L H2O2 Standard	1 mL×1 vial	1 mL×1 vial	5 mL×1 vial	2-8 °C, 12 months, protect from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (580-590 nm, optimum wavelength: 590 nm), Micropipettor, 37 °C incubator

Reagents:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 200 μmol/L H2O2 standard solution with double distilled water to create a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

7. Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma 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 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 200 μL PBS (0.01 M, pH 7.4) 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).

8. The key points of the assay

1. To avoid contamination, aliquot the chromogenic reagent into smaller volumes in EP tubes prior to use.
2. After use, seal the substrate immediately to avoid prolonged exposure to air.
3. Avoid introducing bubbles when adding samples.
4. For optimal accuracy, process no more than 30 sample wells at a time.

9. Operating steps

1. Standard well: Add 20 μL of standard with different concentrations to the standard wells. Sample well: Add 20 μL of sample to the sample wells.
2. Add 200 μL of chromogenic agent to each well.
3. Mix fully with microplate reader for 5 s and measure the OD values of each well at 590 nm with microplate reader, recorded as A1.
4. Add 50 μL of substrate to each well.
5. Mix fully with microplate reader for 5 s and incubate at 37 °C for 5 min. Measure the OD values of each well at 590 nm with microplate reader, recorded as A2. ΔA = A2 - A1.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of the blank (Standard #①) from all standard readings. This is the absolute ΔA value.

3. Plot the standard curve by using absolute ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Liquid sample:

TOS (μmol H2O2 Equiv./L) = (ΔA590 - b) ÷ a × f

2. Tissue and cells sample:

TOS (μmol H2O2 Equiv./gprot) = (ΔA590 - b) ÷ a ÷ C_{pr} × f

[Note]

ΔA590: ΔA_sample–ΔA_blank (ΔA_blank is the ΔA when the standard concentration is 0).

f: Dilution factor of sample before test.

C_{pr}: Protein concentration of sample, gprot/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 (μmol H2O2 Equiv./L)	5.60	34.80	85.00
%CV	2.6	2.2	2.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

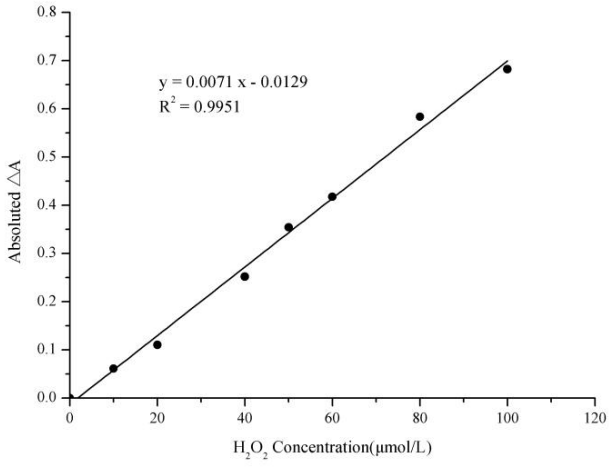
Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol H2O2 Equiv./L)	5.60	34.80	85.00
%CV	3.2	3.6	3.7

Sensitivity

The analytical sensitivity of the assay is 2.5 µmol H2O2 Equiv./L. 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.

Standard curve

As the ΔA 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

Concentration (μmol/L)	0	10	20	40	50	60	80	100
Average ΔA	0.085	0.146	0.195	0.337	0.439	0.502	0.668	0.767
Absolute ΔA	0.000	0.061	0.110	0.252	0.354	0.417	0.584	0.682

12. Appendix II Example Analysis

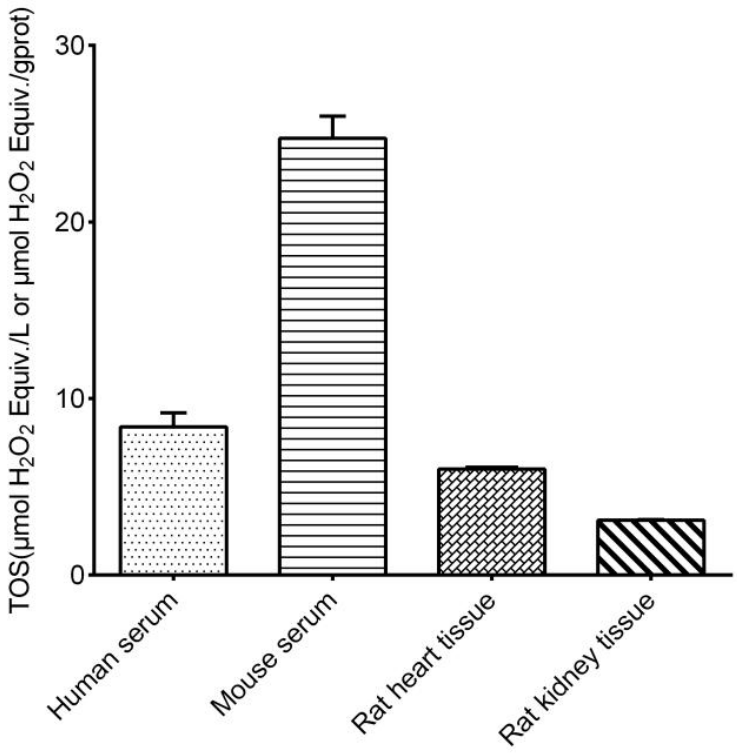
Example analysis:

For human serum, take 20 μL to the sample wells and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.007x - 0.0146, the ΔA value of the sample is 0.142, the average ΔA value of the blank is 0.098, the absolute ΔA value of the sample: ΔA590 = 0.142 - 0.098 = 0.044, and the calculation result is:

TOS (μmol H2O2 Equiv./L) = (0.044 + 0.0146) ÷ 0.007 = 8.37 μmol H2O2 Equiv./L

Detect human serum, mouse serum, 10% rat heart tissue homogenate (the concentration of protein is 8.42 gprot/L), and 10% rat kidney tissue homogenate (the concentration of protein is 11.71 gprot/L) 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.

14. Appendix III Products Cited In Published Literatures

1. Guler A, Yilmaz A, Oncer N, et al. Machine learning-assisted SERS approach enables the biochemical discrimination in Bcl-2 and Mcl-1 expressing yeast cells treated with ketoconazole and fluconazole antifungals[J]. Talanta, 2024, 276. DOI:10.1016/j.talanta.2024.126248.
2. Oi-Jurjevi B, Borkovi-Miti S, Pavlovi S, et al. Lemon Flavonoid Extract Eriomin Improves Pro/Antioxidant Status and Interferes with Cholesterol Metabolism without Affecting Serum Cholesterol Levels in Aged Rats[J]. International Journal of Molecular Sciences, 2024, 25(10). DOI:10.3390/ijms25105221.
3. Prvulovic M, Pavlovic S, Mitic S B, et al. Mitigating the effects of time in the heart and liver: The variable effects of short- and long-term caloric restriction[J]. Mechanisms of Ageing and Development, 2024, 222. DOI:10.1016/j.mad.2024.111992.
4. Bozkurt A S, Tilmaz S G. Ferroptotic Potency of ISM1 Expression in the Drug-Induced Alzheimer's Disease-Like Phenotype Under the Influence of Betulin[J]. Journal of Alzheimer's disease: JAD, 2023, 96(4):1565-1578. DOI:10.3233/JAD-230940.
5. Ayenur Güler, Yardmc B K, Zek N I. Human anti-apoptotic Bcl-2 and Bcl-xL proteins protect yeast cells from aging induced oxidative stress[J]. Biochimie [2025-02-10]. DOI: 10.1016/j.biochi.2024.10.009.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.