TECHNICAL MANUAL

Thioredoxin Reductase (TrxR) Activity Assay Kit

- **SKU CODE:** MAES0275
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can be used to measure thioredoxin reductase (TrxR) activity in animal tissue and cell samples.

3. Detection principle

The thioredoxin system is one of the most important antioxidant systems in the body, capable of stabilizing the redox balance of organisms and regulating signal transduction. It is also involved in cellular redox reactions, nucleic acid metabolism, cell growth, and tumorigenesis. Thioredoxin reductase (TrxR) is a NADPH-dependent dimeric selenoenzyme containing the flavin adenine dinucleotide (FAD) domain and is a member of the pyridine nucleotide-disulfide oxidoreductase family. The thioredoxin system consists of NADPH, thioredoxin reductase, and thioredoxin.

The TrxR catalytic substrate enables the reducing agent to reduce the color developer, which has a characteristic absorption peak at 412 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL x 1 vial	50 mL x 1 vial	-20 °C, 12 months
Reagent 2	Substrate A	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 3	Substrate B	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Chromogenic Agent	0.25 mL x 1 vial	0.25 mL x 2 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 (412 nm), 37 °C incubator.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 3 mL buffer solution and mix well. Store at -20 °C for up to 2 days.
3. **Preparation of substrate B working solution:** Dissolve one vial of substrate B with 0.25 mL buffer solution and mix well. Store at -20 °C for up to 2 days.
4. **Preparation of measuring working solution:** For each well, prepare 200 µL of measuring working solution by thoroughly mixing 144 µL of buffer solution, 50 µL of substrate A working solution, 4 µL of substrate B working solution, and 2 µL of chromogenic agent. The measuring working solution should be prepared immediately before use and protected from light.
5. **Preparation of control working solution:** For each well, prepare 200 µL of control working solution by thoroughly mixing 198 µL of buffer solution and 2 µL of chromogenic agent. The control working solution should be prepared immediately before use and protected from light.

7. Sample preparation

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) using a dounce homogenizer at 4 °C.

3. Centrifuge at 10,000 xg for 10 minutes to remove insoluble material. Collect the supernatant and keep it on ice for detection.

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

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation: $1x10^6$ cells).

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

3. Homogenize $1x10^6$ cells in 200 µL normal saline (0.9% NaCl) using an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 xg for 10 minutes to remove insoluble material. Collect the supernatant and keep it on ice for detection.

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

Dilution of sample:

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

10% Mouse liver tissue homogenate: 2-3

10% Mouse kidney tissue homogenate: 2-3

10% Mouse lung tissue homogenate: 2-3

HL-60 cells: 1

CHO cells: 1

HeLa cells: 1

Jurkat cells: 1

Molt-4 cells: 1

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

8. Operating steps

1. Control well: Add 10 µL of sample to the corresponding wells. Sample well: Add 10 µL of sample to the corresponding wells.
2. Add 190 µL of control working solution to control wells, add 190 µL of measuring working solution to sample wells.
3. Mix thoroughly using the microplate reader for 5 seconds and incubate at 37 °C for 15 minutes protected from light.
4. Measure the OD value of each well using a microplate reader at 412 nm.

9. Calculation

For tissue and cell samples:

Definition: The amount of thioredoxin reductase (TrxR) in 1 g tissue or cell sample protein that hydrolyzes the substrate to produce 1 µmol product in 1 minute at 37 °C is defined as 1 unit.

TrxR activity (U/gprot) = $\text{deltaA}_{412} \div (\varepsilon \times d) \times (V_1 \div V_2) \div T \times f \div C_{pr}$

[Note]

deltaA$_{412}$: The change in OD value of sample, $A_{sample} - A_{control}$.

ε: Molar absorption coefficient, 1.36 x10^{-2} L/μmol/cm.

d: Optical path, 0.6 cm.

V_1: The volume of reaction system, 0.2 mL.

V_2: The volume of sample in reaction system, 0.01 mL.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: The time of reaction, 15 min.

10. 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/L)	2.4	18.6	32.5
%CV	2.9	2.8	1.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.4	18.6	32.5
%CV	3.2	5.3	4.1

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 96%.

Recovery

Sample	Sample 1	Sample 2	Sample 3
Expected Conc. (µmol/L)	10.5	22.5	38.3
Observed Conc. (µmol/L)	10.3	21.4	36.4
Recovery rate (%)	98	95	95

Sensitivity

The analytical sensitivity of the assay is 0.82 U/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.

11. Appendix II Example Analysis

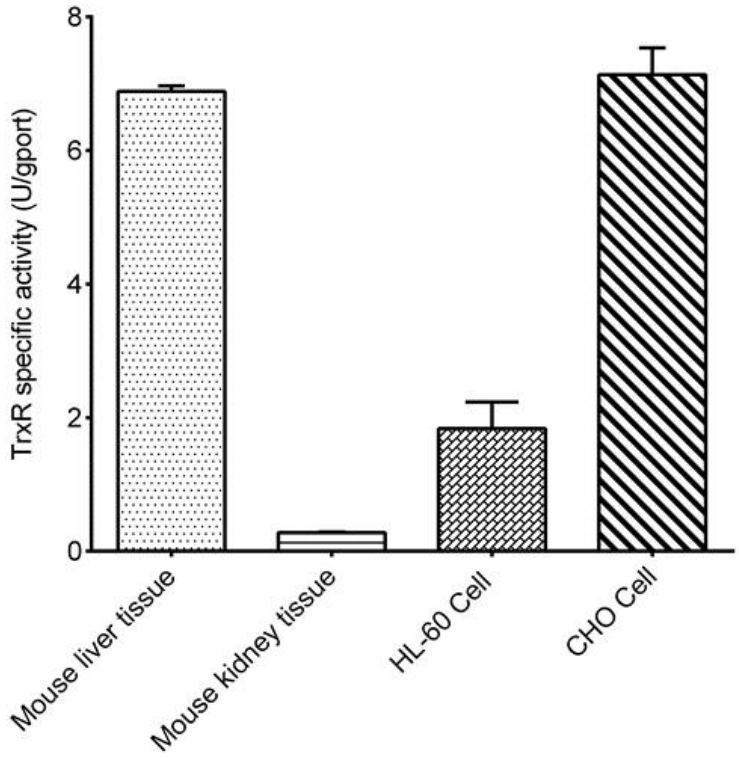
Example analysis:

For 10% mouse liver tissue homogenate, diluted 2-fold, carry out the assay according to the operation steps. The results are as follows:

The OD value of the sample well is 0.881, the OD value of the control well is 0.310, deltaA_{412} = 0.881 – 0.310 = 0.571, the concentration of protein in the sample is 13.56 gprot/L, and the calculation result is:

TrxR activity (U/gprot) = 0.571 ÷ (1.36 x10^{-2} x 0.6) x (0.2 ÷ 0.01) ÷ 15 x 2 ÷ 13.56 = 13.76 U/gprot

Detection of 10% mouse liver tissue homogenate (protein concentration: 13.56 gprot/L), 10% mouse kidney tissue homogenate (protein concentration: 16.78 gprot/L), 6.9x10^{6} HL-60 cells (protein concentration: 1.28 gprot/L), and 1.5x10^{6} CHO cells (protein concentration: 0.191 gprot/L) according to the protocol yields 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 is not within 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.