（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K548-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（412 nm）

Detection range：0.82-46.81 U/L

Elabscience® Thioredoxin Reductase (TrxR) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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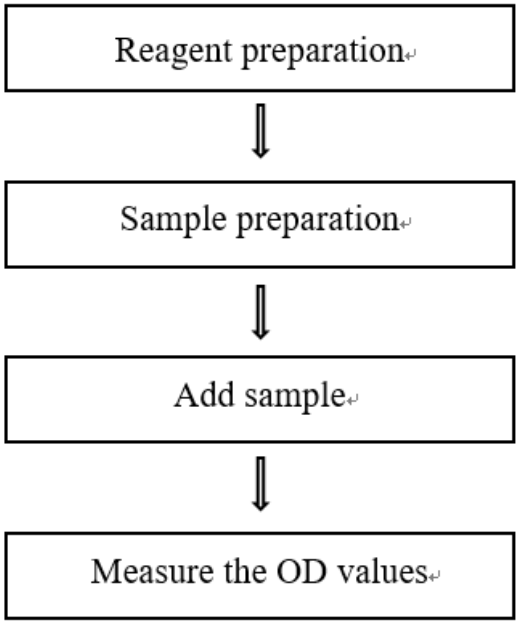
Assay summary



Intended use

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

Detection principle

Thioredoxin system, as one of the most important antioxidant systems in the body, can stabilize the redox balance of organisms, regulate signal transduction. It is also related to cell REDOX reaction, nucleic acid metabolism, cell growth and tumorigenesis. Thioredoxin reductase (TrxR) is a NADPH-dependent dimer selenase 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 makes the reducing agent reduce the color developer with a characteristic absorption peak at 412 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL ×1 vial	50 mL ×1 vial	-20℃, 12 months
Reagent 2	Substrate A	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months shading light
Reagent 3	Substrate B	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months shading light
Reagent 4	Chromogenic Agent	0.25 mL ×1 vial	0.25 mL ×2 vials	-20℃, 12 months shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (412 nm), 37℃ incubator.

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution:
Dissolve a vial of substrate A with 3 mL buffer solution and mix well. Store at -20 ℃ for 2 days.

③ The preparation of substrate B working solution:
Dissolve a vial of substrate B with 0.25 mL buffer solution and mix well. Store at -20 ℃ for 2 days.

④ The preparation of measuring working solution:
For each well, prepare 200 μL of measuring working solution (mix well 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 on spot protected from light.

⑤ The preparation of control working solution:
For each well, prepare 200 μL of control working solution (mix well 198 μL of buffer solution and 2 μL of chromogenic agent). The control working solution should be prepared on spot protected from light.

Sample preparation

① 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) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

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 normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×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 (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
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 do pretest to confirm the dilution factor

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 fully with microplate reader for 5 s and incubate at 37℃ for 15 min protected from light.

④ Measure the OD value of each well with microplate reader at 412 nm.

Calculation

The sample:

The tissue and cell sample:

Definition: The amount of thioredoxin reductase (TxrR) in 1 g tissue or cell sample protein that hydrolyze the substrate to 1 μmol product in 1 minute at 37 oC is defined as 1 unit.

$$\text{TxrR activity (U/gprot)} = \Delta A_{412} \div (\varepsilon \times d) \times (V_1 \div V_2) \div T \times f \div C_{pr}$$

[Note]

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

ε: Molar absorption coefficient, 1.36×10^{-2} L/μmol/cm.

d: Optical path, 0.6 cm.

V_1: The volume 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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	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.

Appendix II Example Analysis

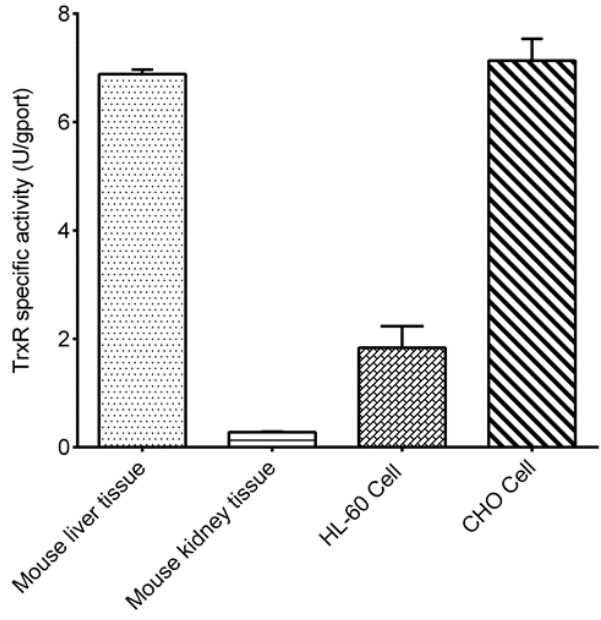
Example analysis:

For 10% mouse liver tissue homogenate, dilute for 2 times, and carry 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, ΔA_{412} = 0.881 – 0.310 = 0.571, the concentration of protein in sample is 13.56 gprot/L, and the calculation result is:

$$\text{TrxR activity (U/gprot)} = 0.571 \div (1.36 \times 10^{-2} \times 0.6) \times (0.2 \div 0.01) \div 15 \times 2 \div 13.56 = 13.76 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 13.56 gprot/L), 10% mouse kidney tissue homogenate (the concentration of mitochondria protein is 16.78 gprot/L), 6.9×10^6 HL-60 cells (the concentration of protein is 1.28 gprot/L), 1.5×10^6 CHO cells (the concentration of protein is 0.191 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
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 taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.