# Oxidized thioredoxin reductase (TrxR) test kit

**(Cat/No.:BC047 Size:50T/48S)**

## 1. Significance of the Assay

TrxR is an NADPH-dependent dimeric selenoenzyme containing a FAD domain, belonging to the pyridine nucleotide–disulfide oxidoreductase family. Together with thioredoxin and NADPH, it constitutes the thioredoxin system. TrxR, similar to GR activity, catalyzes the reduction of GSSG to GSH and is one of the key enzymes in the glutathione redox cycle.

## 2. Principle of the Assay (Spectrophotometric Method)

TrxR catalyzes the reduction of DTNB by NADPH to produce TNB and NADP+. TNB has a characteristic absorption peak at 412 nm. By measuring the rate of increase of TNB at 412 nm, the TrxR activity can be calculated.

## 3. Self-provided Experimental Supplies and Instruments

Visible spectrophotometer, low-temperature centrifuge, adjustable micropipettes, 1 mL glass cuvettes (1 cm path length), distilled water, protein concentration assay kit (for tissue or cell samples).

## 4. Reagents and Preparation (Kit shelf life: 6 months)

**Reagent 1**: Liquid ×1 bottle, store at 4℃.

**Reagent 2**: Liquid ×1 bottle, store at 4℃ protected from light.

**Reagent 3**: Powder ×1 bottle, store at 4℃. Dissolve in 5 mL distilled water before use.

## 5. Crude Enzyme Extraction

1. Tissue: According to the ratio of tissue weight (g) : extraction buffer volume (mL) 1:5~10 (recommend ~0. 1 g tissue with 1 mL Reagent 1), homogenize on ice, centrifuge at 8000g, 4℃ for 10 min, collect the supernatant on ice for assay.
2. Bacteria or Fungi: According to the ratio of cell number ($10^4$) : extraction buffer volume (mL) 500~1000:1 (recommend $5\times10^6$ cells with 1 mL Reagent 1), lyse cells by ice-bath ultrasonication (power 300 W, 3 s on, 7 s off, total 3 min); then centrifuge at 8000g, 4℃ for 10 min, collect the supernatant on ice for assay.
3. Serum (plasma) or other liquid samples: Use directly.

## 6. Procedure

1. Preheat the spectrophotometer for 30 min, set wavelength to 412 nm, zero with distilled water.
2. Preheat Reagent 1 at 25℃ (general species) or 37℃ (mammals) for 30 min.
3. **Blank tube:** Add 100 µL distilled water, 100 µL Reagent 2, 100 µL Reagent 3, **700 µL Reagent 1** into a test tube or centrifuge tube, mix quickly, transfer to a 1 mL cuvette, measure absorbance at 412 nm at 10 s and 310 s, recorded as A1 and A2. ΔA_blank = A2 –

A1. (Only one blank tube is required. )

4. **Test tube:** Add 100 μL Reagent 2, 100 μL Reagent 3, 700 μL Reagent 1, 100 μL supernatant (or serum/plasma) into a clean test tube or centrifuge tube, mix quickly, transfer to a 1 mL cuvette, measure absorbance at 412 nm at 10 s and 310 s, recorded as A3 and A4. ΔA_test = A4 – A3.

## 7. Calculation Formula

### 1. TrxR activity in tissues, bacteria, or cells

**（1）. Calculation based on protein concentration**

**Unit (U) definition:** One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 nmol DTNB per minute per mg of tissue protein at 25℃ or 37℃.

$$\text{TrxR Activity (U/mgprot)} = \frac{\Delta A_{Sample} - \Delta_{Blank}}{\varepsilon \times d} \times \frac{V_{Total}}{V_{Sample} \times Cpr} \div T$$
$$= 147 \times (\Delta A_{Sample} - \Delta_{Blank}) \div Cpr$$

**（2）. Calculation Based on Sample Weight**

**Unit (U) definition:** One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 nmol DTNB per minute per gram of sample at 25℃ or 37℃.

$$\text{TrxR Activity (U/g Fresh Weight)} = \frac{\Delta A_{sample} - \Delta_{blank}}{\varepsilon \times d} \times \frac{V_{Total}}{V_{Sample} \times W \div V_{\substack{Sample \\ Total}}} \div T$$
$$= 147 \times (\Delta A_{sample} - \Delta_{blank}) \div W$$

**（3）. Calculation Based on Cell Number**

**Unit (U) definition:** One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 nmol DTNB per minute per $10^4$ cells at 25℃ or 37℃.

$$\text{TrxR Activity (U/10}^4\text{ cells)} = \frac{\Delta A_{sample} - \Delta_{blank}}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample} \times Cell\ Number \div V_{\substack{Sample \\ Total}}} \div T$$
$$= 147 \times (\Delta A_{sample} - \Delta_{blank}) \div Cell\ Number$$

### 2. Calculation for Liquid Samples

**Unit (U) definition:** One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reduction of 1 nmol DTNB per minute per mL of sample at 25℃ or 37℃.

$$\text{TrxR Activity (U/mL)} = \frac{\Delta A_{sample} - \Delta_{blank}}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample}} \div T$$
$$= 147 \times (\Delta A_{sample} - \Delta_{blank})$$

**ε**:Molar extinction coefficient of TNB at 412 nm, 0. 0136 L/μmol/cm;

**d**:Cuvette path length, 1 cm;

**$V_{total}$**:Total reaction system volume (L), 1000 μL = 0. 001 L;

**Cpr**:Protein concentration of supernatant (mg/mL);

$V_{sample}$:Volume of sample added to the reaction system (mL), 100 μL = 0. 1 mL;
$V_{sample\ total}$:Volume of extraction buffer added, 1 mL;
**W**:Sample weight, g;
**T**:Reaction time, 5 min.

## 8. Precautions

1. Before the assay, 1–2 pretests should be performed to ensure absorbance changes linearly within 5 min. For TrxR activity measurement in mammalian tissues and blood products, the sample generally needs to be diluted about 5-fold with distilled water; operations during the assay must be performed quickly.
2. Reagent 3 should be used as soon as possible after preparation (recommended within 3 days).