Oxidized Thioredoxin Reductase Assay Kit (Microanalysis)

Description

Thioredoxin reductase (Oxidized Thioredoxin Reductase, TrxR) is an NADPH-dependent dimeric selenoenzyme containing an FAD domain and belongs to the family of pyridine nucleotide–disulfide oxidoreductases. Together with thioredoxin and NADPH, it constitutes the thioredoxin system. TrxR has enzymatic activity similar to that of glutathione reductase (GR) and catalyzes the reduction of GSSG to GSH, making it one of the key enzymes in the glutathione redox cycle.

Detection Principle

TrxR catalyzes the NADPH-dependent reduction of DTNB to generate TNB and $NADP^+$. TNB exhibits a characteristic absorption peak at 412 nm. However, reduced glutathione (GSH) can also react with DTNB to produce TNB. Therefore, 2-vinylpyridine is used to inhibit the endogenous reduced glutathione present in the sample. By measuring the rate of increase in TNB absorbance at 412 nm, the activity of TrxR can be calculated.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0136M-A	120 mL x 1	4 °C
CB0136M-B	1 vial (powder) x 1	Store at 4 °C protected from light; before use, dissolve by adding 2 mL of 1× PBS buffer (use within 3 days).
CB0136M-C	1 vial (powder) x 1	Store at 4 °C; before use, dissolve by adding 2 mL of distilled water (use within 3 days).
CB0136M-D	30 μL×1	Store at −20 °C; before use, dilute 10-fold with absolute ethanol according to the number of samples.

Prior to the formal determination, a preliminary assay should be conducted using 2-3 samples with large expected differences.

Instructions

I. Preparation of Lab Instruments

Visible spectrophotometer / microplate reader, micro glass cuvettes / 96-well plates, water bath, balance, drying oven, glass tubes, centrifuge, adjustable pipettes, distilled water, 1× PBS buffer.

II. Extraction of Crude Enzyme

1. Tissues:

Homogenize on ice at a ratio of tissue mass (g) : CB0136M-A volume (mL) = 1 : 5–10

(Recommended: weigh ~0.1 g tissue and add 1 mL CB0136M-A).

Centrifuge at 8000 × g, 4 °C for 10 min. Collect the supernatant and keep on ice for analysis.

2. Bacteria / cells:

Use a ratio of cell number (×10^4 cells) : CB0136M-A volume (mL) = 500–1000 : 1

(Recommended: add 1 mL CB0136M-A to 5 × 10^6 cells).

Lyse cells by ultrasonication on ice (300 W; 3 s on / 7 s off; total 3 min).

Then centrifuge at 8000 × g, 4 °C for 10 min. Collect the supernatant and keep on ice for analysis.

3. Serum and other liquid samples: Measure directly.

III. Assay Procedure

1. Preheat the spectrophotometer for 30 minutes, set the wavelength to 412 nm, and zero the instrument using distilled water.

2. Preheat CB0136M-A at 25 °C (for general species) or 37 °C (for mammals) for 30 minutes. Before measurement, mix the sample supernatant with CB0136M-D at a volume ratio of 50:1 (i.e., add 2 μL of CB0136M-D to 100 μL of supernatant). Incubate in a 37 °C water bath for 30 minutes, then place on ice.

3. Add the following reagents into a micro glass cuvette or a 96-well plate:

	Blank Tube（μL）	Sample Tube（μL）
CB0136M-B	20	
CB0136M-C	20	
CB0136M-A	160	
After rapid mixing, measure the absorbance at 412 nm at 10 s. Then incubate in a 37 °C water bath for 5 min, quickly remove and measure the absorbance at 412 nm again, recorded as A1 and A2. Calculate the blank tube ΔA as ΔA_blank = A2 − A1.		
CB0136M-B		20
CB0136M-C		20
CB0136M-A		140
supernatant		20
After rapid mixing, measure the absorbance at 412 nm at 10 s. Then incubate in a 37 °C water bath for 5 min, promptly remove and measure the absorbance at 412 nm again. Record the two readings as A3 and A4, respectively. Calculate the absorbance change for the assay tube as ΔA = A4 − A3.		

Note: Blank tubes only need to be measured 1–2 times.

IV. Calculation of TrxR Activity

A. Measure using a micro quartz cuvette

1. Based on protein concentration

At 25 °C or 37 °C, the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per milligram of protein is defined as one unit of enzyme activity.

TrxR (U/mg prot) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷(Cpr×V2)÷T= 147×(ΔA sample - ΔA blank)÷Cpr

2. Based on sample mass

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per gram of sample.

TrxR (U/g) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷(W×V2÷V3)÷T = 147×(ΔA sample - ΔA blank)÷W

3. Based on bacterial or cell density

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per 10^4 cells.

TrxR (U/104 cell) = (ΔAsample - ΔA blank)÷ε÷d×109×V1÷(cell number×V2÷V3)÷T= 147×(ΔA sample - ΔA blank)÷cell number

4. Based on Liquid Volume

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per milliliter of liquid.

TrxR (U/mL) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷V2÷T= 147×(ΔA sample - ΔA blank)

Note:

ε: Molar extinction coefficient of TNB at 412 nm, 1.36 × 10^4 L/mol/cm; d: Optical path length of the cuvette, 1 cm

V1: Total volume of the reaction system (L), 200 μL = 2 × 10^4 L; Cpr: Protein concentration of the supernatant (mg/mL), to be determined separately

W: Sample mass; V2: Volume of supernatant added to the reaction system (mL), 20 μL = 0.02 mL
V3: Volume of extraction buffer, 1 mL; T: Reaction time (min), 5 min
Cell number: Expressed in units of 10^4 cells (ten thousand cells); 10^9: Unit conversion factor, 1 mol = 10^9 nmol

B. Measure using a 96-well plate

1. Based on protein concentration

At 25 °C or 37 °C, the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per milligram of protein is defined as one unit of enzyme activity.

TrxR (U/mg prot) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷(Cpr×V2)÷T= 294×(ΔA sample-ΔA blank)÷Cpr

2. Based on sample mass

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per gram of sample.

TrxR (U/g) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷(W×V2÷V3)÷T= 294×(ΔA sample - ΔA blank)÷W

3. Based on bacterial or cell density

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per 10^4 cells.

TrxR (U/104cell) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷(cell number×V2÷V3)÷T= 294×(ΔA sample - ΔA blank)÷cell number

4.Based on Liquid Volume

At 25 °C or 37 °C, one enzyme activity unit is defined as the amount of enzyme that catalyzes the reduction of 1 nmol of DTNB per minute per milliliter of liquid.

TrxR (U/mL) = (ΔA sample - ΔA blank)÷ε÷d×109×V1÷V2÷T= 294×(ΔA sample - ΔA blank)

Note:

ε: Molar extinction coefficient of TNB at 412 nm, 1.36 × 10^4 L/mol/cm; d: Optical path length of the 96-well plate, 0.5 cm
V1: Total volume of the reaction system (L), 200 μL = 2 × 10^4 L; Cpr: Protein concentration of the supernatant (mg/mL), to be determined separately
W: Sample mass; V2: Volume of supernatant added to the reaction system (mL), 20 μL = 0.02 mL
V3: Volume of extraction buffer, 1 mL; T: Reaction time (min), 5 min
Cell number: Expressed in units of 10^4 cells (ten thousand cells); 10^9: Unit conversion factor, 1 mol = 10^9 nmol

Precautions

1. Before formal measurement, perform a preliminary test using 1–2 samples to ensure that the absorbance shows a linear change within 5 minutes. For TrxR activity assays of mammalian tissues and blood products, samples generally need to be diluted approximately 5-fold with distilled water. All procedures during the assay should be carried out rapidly.
2. Since CB0136M-A contains a certain concentration of protein (approximately 0.1 mg/mL), the protein contributed by the extraction buffer itself must be subtracted when determining the protein concentration of the samples.
3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.