CheKine™ Micro Thioredoxin Reductase (TrxR) Assay Kit

Cat #: KTB1650 Size: 48 T/96 T

	Micro Thioredoxin Reductase (TrxR) Assay Kit		
REF	**Cat #:** KTB1650	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Thioredoxin Reductase (TrxR) is a NADPH-dependent dimer selenase that includes the FAD domain. It belongs to the pyridine nucleotide-disulfide redoxase family and forms a Thioredoxin system with Thioredoxin and NADPH. TrxR has similar activity to GR and catalyzes the reduction of GSSG to GSH, which is one of the key enzymes in the glutathione redox cycle. CheKine™ Micro Thioredoxin Reductase (TrxR) Assay Kit provides a simple method for detecting activity of TrxR in a variety of biological samples such as serum, plasma, animal tissues, cells and bacterium. TrxR can catalyzes the reduction of DTNB by NADPH to generate TNB and $NADP^+$. TNB has a characteristic absorption peak at 412 nm. However, the reduced glutathione can also react with DTNB to form TNB, so this kit uses 2-vinylpyridine to inhibit the reduced glutathione present in samples. TrxR activity can be calculated by measuring the increase rate of TNB at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	50 mL	100 mL	4℃
Chromogen	1	1	4℃, protected from light
TrxR Cofactor	1	1	-20℃, protected from light
Inhibitor	60 μL	120 μL	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Freezing centrifuge, water bath
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Assay Buffer: Ready to use as supplied. Store at 4℃.

Working Chromogen: Powder; Add 3 mL deionized water to dissolve Chromogen before use. Store at 4℃, protected from light.

Working TrxR Cofactor: Powder; Add 5 mL deionized water to dissolve TrxR Cofactor before use. Store at -20℃, protected from light.

Inhibitor： Ready to use as supplied. Equilibrate to room temperature before use. Store at-20℃, protected from light.

Sample Preparation

1. Animal Tissues: Weigh 0.1 g tissues, add 1 mL cold Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Serum and other Liquids: direct determination.
3. Bacteria or Cells: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Assay Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: The whole process needs to be carried out on ice, and the enzyme activity should be determined on the same day, to avoid repeated freeze-thaw of the homogenate solution. In the detection of TrxR activity in cells, the cell number must be between 3-5x10^6, and the extraction of TrxR in cells can be followed by Assay Buffer grinding or ultrasonic treatment, cells cannot be treated by lysate. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.
2. Assay Buffer place at 25℃ (for general species) or 37℃ (for mammals) incubation for 30 min.
3. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Test Well (μL)
Assay Buffer	20	0
Sample	0	20
Inhibitor	1	1
Mix well, incubate for 30 min at 25℃ (for general species) or 37℃ (for mammals)		
Assay Buffer	140	140
Working Chromogen	20	20
Working TrxR Cofactor	20	20

4. Mix well. The absorbance values were measured at 412 nm for 10 s and 310 s. The blank well 10 s is A_1, 310 s is A_2, and the test well 10 s is A_3, 310 s is A_4, calculate $\Delta A_{Blank}=A_2-A_1$, $\Delta A_{Test}=A_4-A_3$.

Note: Blank well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples to ensure that the change in absorbance within 5 min is linear. Generally, mammalian tissue and blood must be diluted 5 times with deionized water. The reaction temperature has influence on the result. Keep the temperature at 25℃ (for general species) or 37℃ (for mammals). Since Assay Buffer contains a certain concentration of protein (about 0.1 mg/mL), it is necessary to subtract the Assay Buffer itself concentration of protein,

when determining the concentration of protein in the sample.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: at 25℃ or 37℃, 1 μmol DTNB reduction per milligram of protein per minute was catalyzed.

TrxR (U/mg prot)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\div(Cpr\times V_{Sample})\div T$**=0.294×($\Delta A_{Test}-\Delta A_{Blank}$)÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: at 25℃ or 37℃, 1 μmol DTNB reduction per gram of sample per minute was catalyzed.

TrxR (U/g)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Tota}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$**=0.294×($\Delta A_{Test}-\Delta A_{Blank}$)÷W**

3. By cells or bacteria number

Active unit definition: at 25℃ or 37℃, 1 μmol DTNB reduction per 10^4 cells of sample per minute was catalyzed.

TrxR (U/10^4)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$**=0.294×($\Delta A_{Test}-\Delta A_{Blank}$)÷500**

4. By liquid volume

Active unit definition: at 25℃ or 37℃, 1 μmol DTNB reduction per mL of sample per minute was catalyzed.

TrxR (U/mL)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^6\div V_{sample}\div T$**=0.294×($\Delta A_{Test}-\Delta A_{Blank}$)**

Where: $\Delta A_{Blank}=A_2-A_1$, $\Delta A_{Test}=A_4-A_3$; ε: TNB molar extinction coefficient at 412nm, 0.0136 L/μmol/cm; d: 96-well plate light path, 0.5 cm; V_{Total}: Total volume of reaction system, 200 μL=2×10^{-4} L; 10^6:1 mol=1×10^6 μmol; Cpr: Protein concentration of supernatant, mg/mL; W: Sample mass; V_{Sample}: Volume of supernatant added to the reaction system, 20 uL= 2×10^{-2} mL; $V_{Sample\ Total}$: Volume of extraction solution, 1 mL; T: Reaction time, 5 min; 500: Number of cells or bacteria, 5×10^6.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation

Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit
KTB1620	CheKine™ Micro Glutathione Reductases (GR) Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Assay Kit
KTB1660	CheKine™ Micro Thioredoxin Peroxidase (TPX) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.