CheKine™ Micro Thioredoxin Peroxidase (TPX) Assay Kit

Cat #: KTB1660 Size: 48 T/96 T

	Micro Thioredoxin Peroxidase (TPX) Assay Kit		
REF	**Cat #:** KTB1660	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 Peroxidase (TPX) belongs to the family of peroxidases. It mainly achieves anti-oxidation by reducing hydrogen peroxide and some hydroperoxides in the body. It functions similarly to GPX and is one of the key enzymes in the glutathione redox cycle. TPX is ubiquitous in various organisms, such as yeast, plants, animals, protozoa, parasites, bacteria, and archaea, and is highly conservative in evolution. TPX is closely related to the regulation of cell proliferation, differentiation, apoptosis and tumorigenesis. The main functions of TPX include cell detoxification, anti-oxidation, and regulation of signal transduction and immune response mediated by hydrogen peroxide. CheKine™ Micro Thioredoxin Peroxidase (TPX) Assay Kit provides a simple method for detecting TPX in a variety of biological samples, such as serum, plasma, animal tissues, cells, bacteria. TPX catalyzes H_2O_2 to oxidize dithiothreitol (DTT). The absorption wavelength of H_2O_2 is 240nm. By measuring the decrease rate of absorbance at 240 nm, TPX activity can be calculated by the total activity subtracting H_2O_2 catalyzed by catalase (CAT). Therefore, this kit can measure TPX and CAT activities of samples simultaneously.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	50 mL	100 mL	Room temperature
Substrate	10 mL	20 mL	-20℃, protected from light
H_2O_2	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 240 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, incubator
- Deionized water

• Homogenizer (for tissue samples)

Reagent Preparation

Assay Buffer: Ready to use as supplied. Stored at room temperature.

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

H_2O_2： Ready to use as supplied. Equilibrate to room temperature. Store at 4℃, protected from light.

Sample Preparation

1. Animal tissues: Weigh 0.1 g tissues, add 1 mL Assay Buffer and homogenize on ice. Centrifuge at 10,000 rpm for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. 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 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum or plasma: Tested directly.

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 TPX activity in cells, the cell number must be between 3-5×10^6, and the extraction of TPX 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 ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 240 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Assay Buffer and Substrate place at 25℃ (for general species) or 37℃ (for mammals) incubation for 30 min.
3. Add 4 μL supernatant to each well, then add 180 μL Assay Buffer (CAT activity measurement well) or 180 μL of Substrate (total measurement well), and finally add 16 μL H_2O_2. Tap the plate gently to mix the components thoroughly.
4. The absorbance values were measured at 240 nm for 10 s and 130 s. The CAT activity measurement well is recorded as A_1 and A_2, and the Total activity measurement well are recorded as A_3 and A_4.

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 UV plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: at 25℃ or 37℃, 1 nmol H_2O_2 degradation per mL of protein per min was catalyzed.

CAT activity (U/mg prot)=$(A_1-A_2)\div(\varepsilon\times d)\times V_{Total}\div(Cpr\times V_{Sample})\div T$**=1,147×$(A_1-A_2)\div$Cpr**

Total activity (U/mg prot)=$(A_3-A_4)\div(\varepsilon\times d)\times V_{Total}\div(Cpr\times V_{Sample})\div T$**=1,147×$(A_3-A_4)\div$Cpr**

TPX activity (U/mg prot)**=Total activity-CAT activity**

2. Calculated by sample fresh weight

Active unit definition: at 25℃ or 37℃, 1 nmol H_2O_2 degradation per gram of sample per min was catalyzed.

CAT activity (U/g)= $(A_1-A_2)\div(\varepsilon\times d)\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$**=1,147×$(A_1-A_2)\div$W**

Total activity (U/g)=$(A_3-A_4)\div(\varepsilon\times d)\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$**=1,147×$(A_3-A_4)\div$W**

TPX activity (U/g)**=Total activity-CAT activity**

3. By cells or bacteria number

Active unit definition: at 25℃ or 37℃, 1 nmol H_2O_2 degradation per 10^4 cells or bacteria of sample per min was catalyzed.

CAT activity (U/10^4)=(A_1-A_2)÷(ε×d)×V_{Total}÷(500×V_{Sample}÷$V_{Sample\ Total}$)÷T=**2.294×(A_1-A_2)**

Total activity (U/10^4)=(A_3-A_4)÷(ε×d)×V_{Total}÷(500×V_{Sample}÷$V_{Sample\ Total}$)÷T=**2.294×(A_3-A_4)**

TPX activity (U/10^4)**=Total activity-CAT activity**

4. By liquid volume

Active unit definition: at 25℃ or 37℃, 1 nmol H_2O_2 degradation per mL of liquid per min was catalyzed.

CAT activity (U/mL)=(A_1-A_2)÷(ε×d)×V_{Total}×106÷V_{Sample}÷T=**1,147×(A_1-A_2)**

Total activity (U/mL)=(A_3-A_4)÷(ε×d)×V_{Total}×106÷V_{Sample}÷T=**1,147×(A_3-A_4)**

TPX activity (U/mL)**=Total activity-CAT activity**

Where: ε:H_2O_2 molar extinction coefficient, 43600 L/mol/cm = 0.0436 mL/nmol/cm; d: light diameter of 96-well UV plate, 0.5 cm; V_{Total}: total volume of reaction system, 200 uL=0.2 mL; Cpr: protein concentration of supernatant, mg/mL; W: sample mass, g; V_{Sample}: volume of supernatant added to the reaction system, 4 μL= 4×10^{-3} mL; $V_{Sample\ Total}$: volume of extraction solution, 1 mL; T: reaction time, 2 min; 500: number of cells or bacteria, 5×10^6.

B. Microquartz 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
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Assay Kit

Disclaimer

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