Thioredoxin peroxidase (TPX) test kit

（Cat/No.:BC049　　Size:100T/48S）

1. Measurement Significance

TPX belongs to the peroxidase family. In vivo, it exerts antioxidant activity primarily by reducing hydrogen peroxide and certain hydroperoxides. Sharing similar functions with GPX, TPX is also one of the key enzymes in the glutathione redox cycle. Ubiquitously distributed in various organisms—including yeast, plants, animals, protozoa, parasites, bacteria, and archaea—TPX is highly conserved evolutionarily. It is closely associated with the regulation of cell proliferation, differentiation, apoptosis, and tumorigenesis. The main functions of TPX include cellular detoxification, antioxidation, and modulation of hydrogen peroxide-mediated signal transduction and immune responses.

2. Determination principle (Spectrophotometric method)

TPX catalyzes the oxidation of dithiothreitol (DTT) by hydrogen peroxide (H_2O_2). Given that H_2O_2 has an absorption wavelength of 240 nm, the activity of TPX can be calculated by determining the decrease rate of absorbance at 240 nm, with the amount of H_2O_2 decomposed via catalysis by catalase (CAT) subtracted from the control group.

Therefore, this kit can simultaneously determine the activities of TPX and CAT in samples.

3. Self-Provided Instruments & Supplies

Low-temperature Centrifuge, UV-Visible Spectrophotometer, Water Bath, 1 mL Quartz Cuvette, Adjustable Pipette, Distilled Water

4. Composition and Preparation

Reagent 1: Liquid × 1 Bottle, Store at room temperature;

Reagent 2: Liquid × 1 Bottle, Store at -20°C;

Reagent 3: Liquid × 1 Vial, Store at 4°C.

5. Crude Enzyme Extraction

1.Tissues: Homogenize the tissue in an ice bath at a ratio of tissue mass (g) : Reagent 1 volume (mL) = 1 : 5~10 (recommended: weigh approximately 0.1 g of tissue and add 1 mL of Reagent 1). Centrifuge at 10,000 × g for 10 minutes at 4°C, then collect the supernatant and place it on ice for subsequent determination.

2.Bacteria/Fungi: Mix the cells with Reagent 1 at a ratio of cell count (10^4 cells) : Reagent 1 volume (mL) = 500~1000 : 1 (recommended: add 1 mL of Reagent 1 to 5 × 10^6 cells). Perform ultrasonic disruption in an ice bath (power: 300 W, sonication time: 3 s, interval: 7 s, total time: 3 min). Centrifuge at 1,000 × g for 10 minutes at 4°C, then collect the supernatant and place it on ice for subsequent determination.

6. TPX Determination Steps

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 240

nm, and zero it with distilled water;

2. Preheat Reagent 1 and Reagent 2 at 37°C (for mammals) or 25°C (for other species) for more than 30 minutes;

3. CAT Activity Assay Tube: Take a 1 mL quartz cuvette, add 20 μL of supernatant, 900 μL of Reagent 1, and 80 μL of Reagent 3. Mix thoroughly immediately, then measure the absorbance at 240 nm at 10 s and 130 s (denoted as A1 and A2 respectively). Calculate ΔA_{Cat} = A1 - A2.

4. Total Activity Assay Tube: Take a 1 mL quartz cuvette, add 20 μL of supernatant, 900 μL of Reagent 2, and 80 μL of Reagent 3. Mix thoroughly immediately, then measure the absorbance at 240 nm at 10 s and 130 s (denoted as A3 and A4 respectively). Calculate ΔATotal = A3 - A4.

Note: For a large number of samples, each sample requires separate determination of CAT activity.

7. TPX activity calculation

(1) Calculate based on the protein concentration of the sample

Definition of Activity Unit: One unit (1 U) is defined as the amount of enzyme that catalyzes the decomposition of 1 μmol of H2O2 per milligram of protein per minute at 37°C or 25°C.

CAT activity (U/mg prot) = $\triangle A_{CAT} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (Cpr \times V_{Sample}) \div T$

$= 0.573 \times \triangle A_{CAT} \div Cpr$

Total activity (U/mg prot) = $\triangle A_{Total} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (Cpr \times V_{Sample}) \div T$

$= 0.573 \times \triangle A_{Total} \div Cpr$

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

(2) Calculate based on the sample mass

Definition of Activity Unit: One unit (1 U) is defined as the amount of enzyme that catalyzes the decomposition of 1 μmol of H2O2 per gram of tissue per minute at 37°C or 25°C.

CAT activity (U/g) $\triangle A_{CAT} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (W \times V_{Sample} \div V_{Sample\ total}) \div T$

$= 0.573 \times \triangle A_{CAT} \div W$

Total activity (U/g) = $\triangle A_{Total} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (W \times V_{Sample} \div V_{Sample\ total}) \div T$

$= 0.573 \times \triangle A_{Total} \div W$

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

(3) Calculate based on the cell count

Definition of Activity Unit: One unit (1 U) is defined as the amount of enzyme that catalyzes the decomposition of 1 μmol of H2O2 per 104 cells per minute at 37°C or 25°C.

CAT activity (U/10^4 cells) = $\triangle A_{CAT} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (cell\ count \times V_{Sample} \div V_{Sample\ total}) \div T$

$= 0.573 \times \triangle A_{CAT} \div cell\ count$

Total activity (U/10^4 cells) = $\triangle A_{Total} \div (\varepsilon \times d) \times V_{Total\ reaction} \div (cell\ count \times V_{Sample} \div V_{Sample\ total}) \div T$

$= 0.573 \times \triangle A_{Total} \div cell\ count$

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

ε: The molar extinction coefficient of H2O2 at 240 nm is 4.36 × 104 L/mol/cm = 0.0436 L/μmol/cm;

d: Cell optical path (cm), 1 cm;

$V_{Total\ reaction}$: Overall reaction system(L),1000μL=1×10^{-3}L;

Cpr: Protein concentration of the supernatant (mg/mL) needs to be determined separately; it is recommended to use our company's BCA Protein Quantification Kit;

V_{Sample}: Volume of the supernatant added to the reaction system (mL): 20 μL = 0.02 mL;

$V_{Sample\ total}$: Volume of the extraction solution added: 1 mL;
T: Catalytic reaction time (min): 2 min;
W: Sample mass (g);
Cell count (10^4).