# CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Cat #: KTB1640 Size: 48 T/96 T

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| | **Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit** | | |
| REF | **Cat #:** KTB1640 | 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

Glutathione Peroxidase (GSH-Px) is one of the main enzymes that catalyze the oxidation of reduced glutathione (GSH) in the glutathione redox cycle. GSH-Px not only specifically catalyzes the reaction between reduced glutathione and ROS to form oxidized glutathione GSSG, thereby protecting the biofilm from ROS damage and maintaining the normal function of cells; it also protects the liver and improves the body immunity, antagonizing the harmful effects of harmful metal ions on the body and increasing the body's ability to resist radiation. CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit provides a simple method for detecting activity of GSH-Px in a variety of biological samples such as Serum, Plasma, Animal Tissues, Cells and Bacteria. GSH-Px catalyzes $H_2O_2$ to oxidize GSH to produce GSSG; Glutathione Reductase (GR) catalyzes NADPH to reduce GSSG to regenerate GSH, while NADPH oxidizes to produce $NADP^+$; NADPH has a characteristic absorption peak at 340 nm, while $NADP^+$ does not; NADPH dehydrogenation rate is determined by measuring the decrease rate of absorbance at 340 nm to calculate GSH-Px activity.

## Materials Supplied and Storage Conditions

| Kit components | Size | | Storage conditions |
|---|---|---|---|
| | 48 T | 96 T | |
| Assay Buffer | 50 mL+20 mL | 100 mL+20 mL | 4℃ |
| Substrate | 1 | 1 | -20℃, protected from light |
| GR | 10 μL | 10 μL | 4℃, protected from light |
| $H_2O_2$ | 200 μL | 200 μL | 4℃, protected from light |

## Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Incubator
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

- Freezing centrifuge
- Deionized water
- Dounce homogenizer (for tissue samples)

## Reagent Preparation

**Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

**Working Substrate:** Prepare before use, add 20 mL Assay Buffer to dissolve Substrate, mix well; Store and aliquot the surplus reagent at -20℃, protected from light.

**GR:** Ready to use as supplied. Equilibrate to room temperature before use; Store at 4℃, protected from light.

**Working $H_2O_2$:** Prepare before use, add 21.5 μL $H_2O_2$ and 5 mL deionized water, mix well and use the prepared reagent on the same day; Store at 4℃, protected from light.

**Working Reagent:** Prepare before use, add all GR to dissolved Working Substrate, mix well (run out the same day); Working Reagent is preheated to 25℃ (general species) or 37℃ (mammal) for 30 min.

## Sample Preparation

1. Tissue Samples: Wash Tissue with cold PBS to remove blood as much as possible. Weigh 0.1 g Tissues, add 1 mL 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. If necessary, can be diluted with physiological saline and the result should be multiplied by the dilution factor.
3. Bacteria, Cells: Collect $5\times10^6$ 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 processes need 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 GSH-Px activity in Cells, the Cell number must be between 3-5×10$^6$, and the extraction of GSH-Px in cells can be followed by Assay Buffer grinding or ultrasonic treatment, Cells cannot be treated by lysate. For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).**

## Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Working Regent place at 25℃ (for general species) or 37℃ (for mammals) incubation for 30 min.
3. Add the following reagents to the 96-well UV plate or microquartz cuvette, follow the table below to set the test:

| Reagent | Blank Well (μL) | Test Well (μL) |
|---|---|---|
| Deionized Water | 20 | 0 |
| Sample | 0 | 20 |
| Working Reagent | 160 | 160 |
| Working $H_2O_2$ | 20 | 20 |

4. Mix well. The absorbance values were measured at 340 nm for 10 s and 70 s. The absorbance value of blank well 10 s is $A_1$, 70 s is $A_2$; the absorbance value of test well 10 s is $A_3$, 70 s is $A_4$, calculate $\Delta A_{Blank}=A_1-A_2$, $\Delta A_{Test}=A_3-A_4$.

**Note: Blank Well only need to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do**

**a pre-experiment with 2-3 samples. The reaction temperature has influence on the result. Keep the temperature at 25℃ (for general species) or 37℃ (for mammals).**

## 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. Use 96-well UV plate calculation formula as below:

(1) By protein concentration

Active unit definition for GSH-Px: at 25℃ or 37℃, 1 μmol NADPH oxidation per milligram of protein per minute was catalyzed.

GSH-Px activity (U/mg prot)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^6]\div(Cpr\times V_{Sample})\div T$**$=3.216\times(\Delta A_{Test}-\Delta A_{Blank})\div Cpr$**

(2) By sample fresh weight

Active unit definition for GSH-Px: at 25℃ or 37℃, 1μmol NADPH oxidation per gram of sample per minute was catalyzed.

GSH-Px activity (U/g)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^6]\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$**$=3.216\times(\Delta A_{Test}-\Delta A_{Blank})\div W$**

(3) By number of cells or bacteria

Active unit definition for GSH-Px: at 25℃ or 37℃, 1 μmol NADPH oxidation per $10^4$ cells or bacteria of sample per minute was catalyzed.

GSH-Px activity (U/$10^4$)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^6]\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$**$=3.216\times(\Delta A_{Test}-\Delta A_{Blank})\div 500$**

(4) By liquid volume

Active unit definition for GSH-Px: at 25℃ or 37℃, 1 μmol NADPH oxidation per mL of liquid per minute was catalyzed.

GSH-Px activity (U/mL) = $[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^6]\div V_{Sample}\div T$**$=3.216\times(\Delta A_{Test}-\Delta A_{Blank})$**

Where: $\Delta A_{Blank} = A_1-A_2$, $\Delta A_{Test}=A_3-A_4$; ε: NADPH molar extinction coefficient $6.22\times10^3$ L/mol/cm; d: 96-well UV plate light path, 0.5 cm; $V_{Total}$: Total volume of reaction system, 200 μL=$2\times10^{-4}$ L; $10^6$:1 mol=$1\times10^6$ μmol; Cpr: Protein concentration of supernatant (mg/mL); W: sample mass, g; $V_{Sample}$: Volume of supernatant added to the reaction system, 20 μL= $2\times10^{-2}$ mL; $V_{Sample\ Total}$: Volume of extraction solution, 1 mL; T: Reaction time, 1 min; 500: Number of cells or bacteria, $5\times10^6$.

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 |
|---|---|
| KTB1600 | CheKine™ Micro Reduced Glutathione (GSH) Assay Kit |
| KTB1610 | CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit |
| KTB1620 | CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit |
| KTB1630 | CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit |
| KTB1650 | CheKine™ Micro Thioredoxin Reductase (TrxR) 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.