# GENLISA® Glutathione Peroxidase, GSH-PX Assay

**REF** : KBCA1556

Ver 2.0

**RUO**

Biochemical Assays for the Quantitative Determination of Glutathione Peroxidase, GSH-PX in serum, plasma, Animal Tissues, Cells and Bacteria

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| **RUO** | **For Research Use Only** | **REF** | **Catalog Number** |
|  | **Store At** | **LOT** | **Batch Code** |
|  | **Manufactured By** |  | **Biological Risk** |
|  | **Expiry Date** |  | **Consult Operating Instructions** |

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**REF** KBCA1556 Σ 100 tests

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**Introduction:**

Glutathione peroxidase (GSH-PX) is a widespread, important hydrogen peroxidase in organisms. It catalyzes reduced glutathione's reduction reaction with peroxide diagnostically leads to protection of cell structure and functions. GSH-PX's active site is Se-cysteine, selenium is necessary part of GSH-PX, 1 mol GSH-PX contains 4 mol selenium. GSH-PX activity can be used as a biochemical indicator of Se level in vivo.

**Intended Use:**

GENLISA® Glutathione Peroxidase, GSH-PX Assay is intended for Quantitative determination of Glutathione Peroxidase, GSH-PX in Serum, Plasma, Animal Tissues, Cells and Bacteria.

**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. This 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 H2O2 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 Provided:**

1. Reagent I - 120 ml x 1 vial
2. Reagent II (Powder) – 2 vials
3. Reagent III (Powder) – 2 vials
4. Reagent IV (Powder) – 200 ul x 1 vial

**Materials & Equipment Required But Not Provided:**

1. Microplate Reader or Ultraviolet Spectrophotometer capable of measuring absorbance at 340 nm
2. Incubator, Ice Maker, Freezing Centrifuge
3. 96-well UV Plate or Microquartz cuvette, precision Pipettes, disposable Pipette Tips
4. Deionized Water
5. Homogenizer (for Tissue Samples)

**Handling/Storage:**

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. After receiving the kit, an unopened kit can be stored at -20ºC for 12 months, protected from light. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

**Sample Preparation:**

1. Animal and Plant Tissues: Weigh 0.1 g Tissues, add 1 ml Reagent I and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4ºC. 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 or 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 Reagent I 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 4ºC. Use supernatant for assay, and place it on ice to be tested.

**Reagent Preparation:**

**Reagent I:** Ready to use. Equilibrate to room temperature before use. Store at 4°C protected from light.

**Working Reagent:** Take a bottle of Reagent II, add 10ml Reagent I to dissolved, mix well. Take a small amount of the mixture in a bottle of Reagent III, mix thoroughly, then transfer back to the bottle of Reagent II, mix for use. (use the prepared reagent on the same day.)

**Reagent IV:** Add 21.5 ul Reagent IV and 5 ml deionized water, mix well, prepare before use, and use the prepared reagent on the same day. Store at 4°C, protected from light.

**Assay Procedure:**

1. Preheat the Microplate Reader or Ultraviolet Spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, Ultraviolet Spectrophotometer deionized water zero.
2. Working Regent and Reagent Ⅳ place at 25ºC (for general species) or 37ºC (for mammals) incubation for 30 min.
3. Sample measurement: Add 20ul Sample, 160ul Working Reagent and 20ul Reagent Ⅳ to the 96-well UV Plate or Microquartz cuvette.Mix Well, the absorbance values were measured at 340nm for 30s and 210s. The absorbance value of 30 s is $A_1$,210 s is $A_2$, calculate $\Delta A=A_1-A_2$.

**Note for protocol:**

1. **In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**
2. **The reaction temperature has influence on the result. Keep the temperature at 25ºC (for general species) or 37ºC (for mammals).**

**Data Analysis**

**A. 96-well UV Plates calculation formula as below**

**(1) By protein concentration**

Active unit definition: at 25ºC or 37ºC, 1 nmol NADPH oxidation per milligram of protein per minute was catalyzed.

GSH-Px activity(U/mg prot)= $[\Delta A\div(\varepsilon\times d)\times V_{Total}\times10^9]\div(Cpr\times V_{Sample})\div T=1072\times\Delta A\div Cpr$

**(2) By sample fresh weight**

Active unit definition: at 25ºC or 37ºC, 1nmol NADPH oxidation per gram of sample per minute was catalyzed.

GSH-Px activity (U/g)= $[\Delta A\div(\varepsilon\times d)\times V_{Total}\times10^9]\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T=1072\times\Delta A\div W$

**(3) By Cells number**

Active unit definition: at 25ºC or 37ºC, 1 nmol NADPH oxidation per $10^4$ cells of sample per minute was catalyzed.

GSH-Px activity (U/$10^4$ Cells)= $[\Delta A\div(\varepsilon\times d)\times VTotal\times10^9]\div(500\times V_{Sample}\div V_{Sample\ Total})\div T=1072\times\Delta A\div500=2.144\times\Delta A$

**(4) By liquid volume**

Active unit definition: at 25ºC or 37ºC, 1 nmol NADPH oxidation per ml of liquid per minute was catalyzed.

GSH-Px activity (U/ml) = $[\Delta A \div (\varepsilon \times d) \times V_{Total} \times 10^9] \div V_{Sample} \div T = 1072 \times \Delta A$

Where:
ε: NADPH molar extinction coefficient $6.22 \times 10^3$ L/mol/cm
d: 96-well plate light path, 0.5cm
$V_{Total}$: Total volume of reaction system, 200ul= $2 \times 10^{-4}$ L
$10^9$:1 mol=$1 \times 10^9$nmol
Cpr: Protein concentration of supernatant , mg/ml
W: sample mass, g
$V_{Sample}$: Volume of supernatant added to the reaction system, 20ul= $2 \times 10^{-2}$ml
$V_{Sample}$ Total: Volume of extraction solution, 1 ml
T: Reaction time, 3 minute
500: Number of cells, $5 \times 10^6$

**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.

**Safety Precautions:**

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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