# Glutathione Peroxidase Activity (GPX) Assay kit

**ZellBio GmbH (Germany)**
**Cat. No : ZB-GPX-A48**
**Cat. No : ZB-GPX-A96**

**Sample Types Validated**

**Serum, Plasma, Cell Culture Supernatant, Tissue Homogenate and Other Related Biological Liquid**

***Please read this insert completely prior to using the product.***

***For Research Use Only. Not For in vitro Diagnostic use***

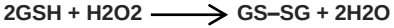
# Related Products

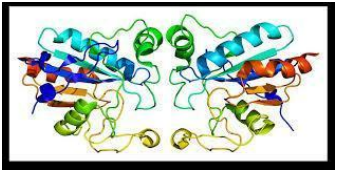
| | ZellBio Antioxidant | Cat No. |
|---|---|---|
| 1 | Total Antioxidant Capacity (TAC) | ZB-TAC-48A/ ZB-TAC-96A |
| 2 | Glutathione Reductase (GR) | ZB-GR-48A/ ZB-GR-96A |
| 3 | Malondialdehyde (MDA) | ZB-MDA-48A/ ZB-MDA-96A |
| 4 | Catalase (CAT) | ZB-CAT-48A/ ZB-CAT-96A |
| 5 | Superoxide Dismutase (SOD) | ZB-SOD-48A/ ZB-SOD-96A |
| 6 | Glutathione (GSH) | ZB-GSH-48A/ ZB-GSH-96A |
| 7 | Glutathione Peroxidase (GPX) | ZB-GPX-48A/ ZB-GPX-96A |
| 8 | Nitric Oxide (NO) | ZB-NO-48A/ ZB-NO-96A |
| 9 | Hydrogen Peroxide ($H_2O_2$) | ZB-HPO-48A/ ZB-HPO-96A |
| 10 | Vitamin C (Vit C) | ZB-VITC-48A/ ZB-VITC-96A |
| 11 | Vitamin E (Vit E) | ZB-VITE-48A/ ZB-VITE-96A |
| 12 | Paraoxonase (POX) | ZB-POX-48A/ ZB-POX-96A |
| 13 | Xanthine Oxidase (XOX) | ZB-XOX-48A/ ZB-XOX-96A |
| 14 | Total Oxidant Status (TOS) | ZB-TOS-48A/ ZB-TOS-96A |
| 15 | Total Polyphenol Content (TPC) | ZB-TPC-48A/ ZB-TPC-96A |

**ZellBio GmbH** assay kit is used to **quantitative** assay Glutathione Peroxidase activity on the basis of the colorimetric assay (**412nm**). **ZellBio GmbH** assay kit, microplate format takes a method with simplified and easy process by our R&D lab techniques. This kit is for research only and is not for use in diagnostic procedures.

## Introduction

**ZellBio GmbH** GPX assay Kit provides a simple, reproducible, and standardized tool for assessment of Glutathione Peroxidase activity in biological sample, e.g. **plasma, serum, tissue homogenates, and cell lysates**. The GPX activity, determine colorimetrically using microplate reader at **412nm**.



## Test principle

**ZellBio GmbH** assay kit uses provides a simple, reproducible, and standardized tool for assessment of Glutathione Peroxidase activity (GPX) in biological sample e.g. plasma, serum, urine, tissue homogenates, and cell lysates. GPX acts as an enzymatic anti-oxidant

defence in extensive physiological actions. Glutathione Peroxidase (GPX) catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. With the exception of phospholipid-hydroperoxide GPX, a monomer, all of the GPX enzymes are tetramers of four identical subunits. Each subunit contains a selenocysteine in the active site which participates directly in the two-electron reduction of the peroxide substrate. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of the selenocysteineBy adding excess GSH, GPX convert it to GSSG and the remain GSH can reduced and generate a yellow color by reducing DTNB (at 412nm). The GPX activity indirectly related to color formation.

## Materials supplied in the Test Kit

| | Kit Contents | 96 Tests | 48 Tests |
|---|---|---|---|
| 1 | Reagent 1 | 20mL | 10mL |
| 2 | Reagent 2 | 5mL | 2.5mL |
| 3 | Reagent 3 | 10mL | 5mL |
| 4 | Reagent 4 / Standard 2mM | Powder | Powder |
| 5 | Reagent 5 | 5mL | 2.5mL |
| 6 | Reagent 6 | 7mL | 3.5mL |
| 7 | Reagent 7 | Powder | Powder |
| 8 | Reagent 8 | 5mL (10X) | 2.5mL (10X) |
| 9 | Microplate (96wells) | 2 | 1 |
| 10 | User Manual | 1 | 1 |

## Materials required but not supplied

1. Microplate reader capable of measuring absorbance at **412nm**,
2. Centrifuge, Micro-pipettes and tips, Vortex mixer and incubator,
3. A source of pure water (Double distilled water),

## Important Notes

1. Before opening the kit kept at the temperature of 2-8˚C, it takes at least 30 minutes to increase naturally to room temperature.

2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.

3. The instruction must be strictly followed while the reading of Microplate reader must be set as the standard of determining the experiment result.

4. Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid

cross contamination.

5. All samples, washing concentration and wastes of every kind should be disposed as infective agent.

6. Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.

## Precision

Intra-assay Precision (Precision within an assay): 3 serum samples with

low, middle and high level GPX activity were tested 12 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 serum samples with low, middle and high level GPX activity were tested on 3 different plates, 10 replicates in each plate.

$$CV\ (\%) = (SD/mean) \times 100$$

Intra-Assay: CV~3.5%

Inter-Assay: CV~4.7%

## Specimen requirements

1. After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20˚C or lower temperature. Avoid repeated freeze-thaw cycles.
2. **Serum**: Allow the serum to clot for 5-10 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
3. **Plasma**: In accordance with the requirements of sample collection, **EDTA / sodium citrate / Heparin** can be used as anti-coagulation. Add EDTA or sodium citrate and mix them for a minutes. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
4. **Cell Culture Supernatant**: Collect by sterile tubes when examining

secrete components. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

**Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (100mM, pH 7.4) for homogenization or freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8˚C and add a certain amount of PBS (pH 7.4) and then homogenize the sample (~100 mg tissue per 1 mL PBS buffer) thoroughly by hand or homogenizer. Centrifuge (at 4000-6000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use. Alternative method for tissue preparation is: Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin to remove any red blood cells and clots. Homogenize the tissue in 5-10 ml of cold buffer (i.e., 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT) per gram tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

## Assay Range

**ZellBio GmbH** GPX assay kit can be used for GPX activity determination in the range of 20-500U/ml.

## Assay Sensitivity

**ZellBio GmbH** GPX activity assay kit can determine GPX in biological samples with 5U/mL sensitivity (5KU/L). In this assay, GPX activity unit was considered as the amount of the sample that will catalyze decomposition of 1 µmole of GSH to GSSG in one minute.

## Reagent preparation

1) For 96 tests kit, dissolve R4 powder in dd-water and make to 10mL. For 48 tests kit, dissolve R4 powder in dd-water and make to 5mL. This is GSH standard solution (2mM).

2) For 96/48 tests kits, R8 10X solution must be diluted 1:10 in dd-water.

3) For 96 tests kit, dissolve R7 powder in ready R8 solution (1X) and make to 44mL. For 48 tests kit, dissolve R7 powder in ready R8 solution (1X) and make to 22mL.

# Assay Protocol

**Each sample will use as itself control**

| | Test Tubes | Blank Tube | Control Tube |
|---|---|---|---|
| Sample (µL) | 125 | - | - |
| dd-water (µL) | - | 125 | - |
| R1 (µL) | 80 | 80 | 80 |
| R2 (µL) | 20 | 20 | 20 |
| R3 (µL) | 40 | 40 | 40 |
| R4 (µL) | 40 | 40 | 40 |
| R5 (µL) | 20 | 20 | 20 |
| Incubate at 37°C for 5 min | | | |
| Sample (µL) | - | - | 125 |
| R6 (µL) | 30 | 30 | 30 |

Mix well and centrifuge at 4000rpm for 10min (**Samples and control supernatants prepared at this step**)

| | Blank | Standard | Control | Tests |
|---|---|---|---|---|
| Supernatant (µL) | 10 | | 10 | 10 |
| R4 (As GSH Standard 2mM) (µL) | | 10 | | |
| R7 (µL) | 200 | 200 | 200 | 200 |

Incubate 5min at room temperature. Read the absorbance with microplate reader/ELISA reader at 412nm and calculate GPX

activity in unknown samples based on below formula:

## Calculation

$$GPX\ activity\,(U/mL) = (OD_{control} - OD_{sample})/(OD_{Standard} - OD_{blank}) \times 6000$$

$$e.g. OD_{control} = 0.133 \text{ and } OD_{sample} = 0.119 \text{ and } OD_{Standard} = 0.426 \text{ and } OD_{blank} = 0.097$$

$$GPX\ activity\,(U/mL) = (0.133 - 0.119)/(0.426 - 0.097) \times 6000 = 258$$

**Note:** Diluted samples (e.g. RBC lysed) must be multiply in dilution factor.

## References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2. Beutler E, Duron O, Kelley BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963 May; 61:882-8.

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