GLUTATHIONE PEROXIDASE

UV Method **50 Tests**

For Research Use Only

INTRODUCTION :

Cellular glutathione peroxidase (GPx) is a member of a family of GPx enzyme whose function is to detoxify peroxides in the cell. Because peroxides can decompose to form highly reactive radicals, the GPx enzymes play a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. The GPx enzymes catalyze the reduction of H_2O_2 to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (R-O-H) using glutathione (GSH) as a source of reducing equivalents:

$$\text{R-O-O-H} + 2\,\text{GSH} \xrightarrow{\text{GPx}} \text{R-O-H} + \text{GSSG} + H_2O$$

With the exception of phospholipid-hydroperoxide GPx, a monomer, all of the GPx enzymes are comprised of 4 identical subunits (monomer Mr 22-23 kDa). Each subunit contains a molecule of selenocysteine in the enzyme active site. The selenocysteine is thought to participate directly in electron donation to the peroxide substrate and become oxidized in the process. The enzyme then uses glutathione as an electron donor to regenerate the reduced form of the selenocysteine. The GPx enzymes accept a wide variety of organic peroxides as substrates. However, with the exception of phospholipid hydroperoxide GPx and perhaps pl.GPx, the enzymes exhibit a strong preference for glutathione as a source of reducing equivalents. Phospholipid-hydroperoxide GPx (Mr 19 kDa) is the only enzyme with significant activity on esterified phospholipids and cholesterol in membranes.

PRINCIPLE :

The assay is an indirect measure of the activity of c-GPx. Oxidized glutathione (GSSG), produced upon reduction of an organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR):

$$\text{R-O-O-H} + 2\,\text{GSH} \xrightarrow{\text{c - GPx}} \text{R-O-H} + \text{GSSG} + H_2O$$

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GR}} 2\text{GSH} + \text{NADP}^+$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (A_{340}) providing a spectrophotometric means for monitoring GPx enzyme activity. The molar extinction coefficient for NADPH is 6220 M^{-1} cm^{-1} at 340 nm. To assay c-GPx, a cell or tissue homogenate is added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A_{340} is recorded. The rate of decrease in the A_{340} is directly proportional to the GPx activity in the sample.

REAGENTS:

1-	Assay Buffer, pH 7.0 Phosphate Buffer Triton X-100	 50 mM 0.1 %
2-	NADPH Reagent (lyopholized) Glutathione (GSH) Glutathione Reductase β-nicotinamide-adenine dinucleotide phosphate reduced (NADPH)	 24 µmol ≥ 12 U 4.8 µmol
3-	Substrate Hydrogen peroxide, (dilute 100 times before use)	

PROCEDURE :

- **Buffer (R1): Content ready for use. Stable up to the expiry date when stored at +2 to +8°C.**
- **Reconstitute the content in the vial (R2) with 5 ml Buffer (R1), Stable for one week at -20°C.**
- **Dilute (R3) 100 times immediately before use . (0.1 ml + 10 ml d. Water) . Discard after use .**

	Sample ml
Buffer (R1)	**1.0**
NADPH (R2)	**0.1**
Sample	**0.01**
H_2O_2 (R3)	**0.1**

Mix well. Record the decrease of absorbance at 340 nm/ min. (A_{340} / min.) over a period of 3 min. against deionized water. The starting absorbance at 340 should not exceed 1.5 and the A_{340}/min. should not exceed 0.05, this can be controled by convenient sample dilution.

CALCULATION :

1. **Optain the change in obsorbance per min (Δ A 340 nm / min).**
2. **Convert the net A_{340}/min for the sample to NADPH consumed (nmol/min/mL) using the following relationship:**

$$\text{1 mU/mL = 1 nmol NADPH/min/mL} = \frac{A_{340}\text{ / min.}}{0.00622}$$

3. **Correct for the dilution of the sample:**
   - **Dilution of sample prior to adding to the cuvette.**
   - **Dilution in the assay**

$$= \frac{\text{Total Volume}}{\text{Volume of Sample}} = \frac{1.21}{0.01} = 121$$

4. **Express the units of activity :**

A- in case of erythrocyte lysate

$$\text{Enzyme Activity (mU / mL)} = \frac{A_{340}\text{ / min.}}{0.00622} \times 121 \times \text{dil. Factor}$$

B- in case of tissue homogenate

$$\text{Enzyme Activity (U/gT)} = \frac{A_{340}\text{ / min.}}{0.00622} \times 121$$

SAMPLE PREPARATION

- **It is advisable to homogenize cells or tissues in buffer containing a freshly added reducing agent to maintain GPx enzyme activity. For homogenization buffers, it is recommended that 2-mercaptoethanol or dithiothreitol be added at a final concentration of 1 mM. Buffers should be freshly made and used the same day. If homogenates will not be assayed immediately, they should be stored at -70ºC.**
- **There is abundant c-GPx in erythrocytes. Red blood cells should be washed from tissue samples by perfusion with isotonic saline prior to homogenization. Prior to dissection, animal tissues (brain, Kidney, liver, etc.) should be perfused through the heart with 0.9% NaCl containing 0.16 mg/mL heparin.**
- **Do not use proteolytic enzymes to remove cells from tissue culture plastic. Remove adherent cells from the dish, plate or flask with a rubber policeman.**

General Sample Preparation Protocol

A Tissue Homogenate

1. **Homogenize the sample in 4-8 volumes (per weight tissue) of cold buffer (e.g., 50 mM phosphate buffer, pH 7.0, containing 5 mM EDTA and 1 mM 2-mercaptoethanol)**
2. **Centrifuge to 4000 rpm for 10-20 minutes at 2-8ºC.**
3. **Remove the supernatant fluid containing the enzyme.**
4. **Freeze samples at -70°C before use, or store on ice if they will be assayed the same day.**

B Erythrocyte Lysates

1. **Blood should be collected using an anticoagulant such as heparin, citrate or EDTA.**
2. **The red cells should be collected by centrifugation (e.g., 4000 rpm x 10 minutes at 4°C) and the plasma drawn off.**
3. **Wash the cells once with for 10 volumes of cold saline,or 4 volums 3 times.**
4. **Lyse the red cell pellets by adding 4 volumes of cold deionized water to the estimated pellet volume.**
5. **Remove the red cell stroma by centrifuging (e.g., 4000 rpm x 10 minutes at 4°C)**
6. **Collect the resulting clarified supernate for use in the assay. Freeze the sample at -70°C before use, or store on ice if they will be assayed the same day.**

REFERENCE :

D.E. Paglia and W. N. Valentine (1967) J. Lab. Clin. Med. 70: 158 – 169.

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

GLUTATHIONE PEROXIDASE

UV Method

R2 at -20ºC **50 Tests**
R1 and R3 at +2 to +8ºC

CAT. No.	**GP 2524**

FOR RESEARCH USE ONLY

REAGENTS

R1	Buffer	**60 mL**
R2	NADPH Lypholized	**1 vial**
R3	Substrate	**1 mL**

CONTACTS

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