



Glutathione Peroxidase Protocol

Version: 1

Replaced by version

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Summary:

Glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxides, including hydrogen peroxides, 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 selenocysteine. The Cayman Chemical Glutathione Peroxidase Assay Kit measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of an organic hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample. The Cayman GPx Assay Kit can be used to measure all of the glutathione-dependent peroxidases in plasma, erythrocyte lysates, tissue homogenates, and cell lysates.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	703102
Buffer		
Standard		
Co-Substrate Mix		
Cumene Hydroperoxide		

Protocol:

1. Background or Non-enzymatic Wells - add 120 μ l of Assay Buffer and 50 μ l of co-substrate mixture to three wells.
2. Positive Control Wells (bovine erythrocyte GPx) - add 100 μ l of Assay Buffer, 50 μ l of Co-Substrate Mixture, and 20 μ l of diluted GPx (control) to three wells.
3. Sample Wells - add 100 μ l of Assay Buffer, 50 μ l of Co-Substrate Mixture, and 20 μ l of sample to three wells. To obtain reproducible results, the amount of GPx added to the well should cause an absorbance decrease between 0.02 and 0.135/min. When necessary, samples should be diluted with Sample Buffer or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 10,000 to bring the enzymatic activity to this level. *NOTE: The amount of sample added to the well should always be 20 μ l. To determine if an additional sample control should be performed see the Interferences section (page 14).*
4. Initiate the reactions by adding 20 μ l of Cumene Hydroperoxide to all the wells being used. Make sure to note the precise time the reaction is initiated and add the Cumene Hydroperoxide as quickly as possible.
5. Carefully shake the plate for a few seconds to mix.
6. Read the absorbance once every minute at 340 nm using a plate reader to obtain at least 5 time points. *NOTE: The initial absorbance of the sample wells should not be above 1.2 or below 0.5.*

1. Determine the change in absorbance (ΔA_{340}) per minute by:
 - a. Plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (a graph is shown on page 13 using bovine erythrocyte GPx) -or-
 - b. Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

$$\Delta A_{340}/\text{min.} = \frac{*| A_{340} (\text{Time 2}) - A_{340} (\text{Time 1}) |}{\text{Time 2 (min.)} - \text{Time 1 (min.)}}$$

*Use the absolute value.

2. Determine the rate of $\Delta A_{340}/\text{min.}$ for the background or non-enzymatic wells and subtract this rate from that of the sample wells.
3. Use the following formula to calculate the GPx activity. The reaction rate at 340 nm can be determined using the NADPH extinction coefficient of $0.00373 \mu\text{M}^{-1}$ *. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP^+ per minute at 25°C .

$$\text{GPx activity} = \frac{\Delta A_{340}/\text{min.}}{0.00373 \mu\text{M}^{-1}} \times \frac{0.19 \text{ ml}}{0.02 \text{ ml}} \times \text{Sample dilution} = \text{nmol/min/ml}$$

*The actual extinction coefficient for NADPH at 340 nm is $0.00622 \mu\text{M}^{-1}\text{cm}^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.6 cm).