

Cellular Glutathione Peroxidase Assay Kit

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CATALOG #

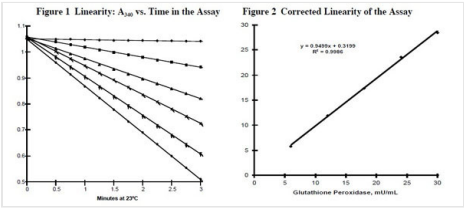
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PRODUCT SYNONYMS

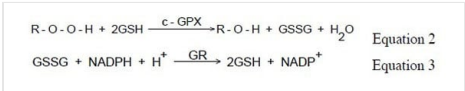
Cellular Glutathione Peroxidase; Cellular Glutathione Peroxidase Assay Kit; Colorimetric Assay for Cellular Glutathione Peroxidase; Cellular Glutathione Peroxidase assay kit

Procedural Notes	Interferences NADPH: enzymes in tissue extracts that consume NADPH can cause a falsely elevated reading in the assay. A blank containing no tert-Butyl Hydroperoxide should be performed to assess nonspecific oxidation of NADPH. High concentrations of reducing agents such as dithiothreitol or mercaptoethanol (0.1 mM or greater final concentration in the assay). It is reported that falsely elevated values of GPx activity will be observed when erythrocyte lysates are assayed due to heme peroxidase activity by hemoglobin.⁷ It has been indicated that there is a negligible contribution to the rate due to hemoglobin in this assay. Therefore, it is not recommended to use Drabkin's reagent (potassium ferricyanide/potassium cyanide) to convert hemoglobin to cyanmethemoglobin in the sample as this can cause inactivation of GPx. Bovine liver Glutathione S-Transferase was tested for interference in this assay. Addition of 17 mU/mL, where 1 unit conjugates 1 μmol of 1-chloro-2,4-dinitrobenzene with reduced glutathione per minute at pH 6.5, 25 degree C, had no effect either on the blank value in the assay (no GPx added) or on a sample containing 30 mU/mL bovine erythrocyte c-GPx. The use of polyethoxy-nonionic detergents (e.g., tween, Triton X-100) is not recommended, as they often contain peroxides. Sample Preparation It is advisable to homogenize cells or tissues in a 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 -70 degree C. 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: 1. Homogenize the sample in 4-8 volumes (per weight tissue) of cold buffer e.g.:50 mM TRIS-HCl, pH 7.5, containing 5 mM EDTA and 1 mM 2-mercaptoethanol. 2. Centrifuge (e.g., 5000-10,000 X g) for 10-20 minutes at 2-8 degree C. 3. Remove the supernatant fluid containing the enzyme. 4. Freeze samples at -70 degree C before use, or store on ice if they will be assayed the same day. 5. Determine the protein concentration of the clarified homogenate to determine the volume to add to the assay. Sample Preparation for Erythrocyte (RBC) Lysates 1. Blood should be collected using an anticoagulant such as heparin, citrate or EDTA. 2. The RBC's should be collected by centrifugation e.g.: 1500 x g for 10 minutes at 4 degree C. 3. Draw off the plasma and buffy coat. 4. Wash the cells once with 10 volumes of cold saline. 5. Lyse the RBC's by adding 4 volumes of cold deionized water to the packed cells. 6. Store on ice if they will be assayed the same day, otherwise, freeze at -70 degree C. 7. Determine hemoglobin concentration of lysate to determine the appropriate dilution (see above). Using the c-GPx Assay on a Microtiter Plate Reader: If a microtiter plate reader capable of measuring A340 is available, the following may be helpful: The molar extinction coefficient, ε, for NADPH at 340 nm is 6220 M⁻¹ cm⁻¹. However, the effective path length in microtiter plate readers may not be 1 cm, therefore, the rate of change in the concentration of NADPH cannot be determined using ε. The researcher must determine a correction factor for the path length in the microtiter plate reader for a specific microtiter plate. A comparison of the rates obtained in a spectrophotometer with a 1 cm path length with those obtained in a microtiter plate using the same samples can be used to determine a correction factor for the path length. The following volumes of reagents and samples would be equivalent to those described for the spectrophotometer: 75 μL Assay Buffer 75 μL NADPH Reagent 15 μL Sample 75 μL 0.007% tert-Butyl Hydroperoxide Limitations 1. The rate of consumption of NADPH is directly proportional to the GPx concentration over a range from 0.035 A340/min up to approximately 0.15 A340/min. a. Samples yielding a rate of change greater than 0.15 A340/min should be further diluted in Assay Buffer and measured again. b. Samples giving a rate of change of less than 0.035 A340/min should be assayed again using smaller dilution (e.g., dilute the stock sample 1/3 or 1/5 prior to assay, instead of 1/10. 2. The temperature of the reaction mixture must be the same each time to avoid variation in the results. 3. The A340 of the NADPH Reagent will slowly decrease with time after reconstitution. If the A340 of a complete assay mixture at t = 0 is ! 0.8, the NADPH Reagent vial in use should be discarded and another vial reconstituted. Assay Performance To ensure reproducibility between assays, a glutathione peroxidase control should be used. This control should be frozen in small volumes, preferably at -70 degree C, in an appropriate buffer (e.g.: 50 mM Tris-HCl, pH 7.2
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IMAGES



Application Data



Application Data



Application Data

ADDITIONAL INFORMATION

Related Product Information for Cellular Glutathione Peroxidase assay kit

Introduction: The Analyte

Cellular glutathione peroxidase (c-GPx, EC 1.11.1.9) is a member of a family of GPx enzymes whose function is to detoxify peroxides in the cell.¹ 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 H₂O₂ to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (R-OH) using glutathione (GSH) as a source of reducing equivalents:

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.¹ The GPx enzymes accept a wide variety of organic peroxides as substrates. However, with the exception of phospholipid hydroperoxide GPx and perhaps pGPx, 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.

Principles Of The Procedure

This assay is an indirect measure of the activity of c-GPx.⁷ 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):

The oxidation of NADPH to NADP + is accompanied by a decrease in absorbance at 340 nm (A₃₄₀) 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, tert-butyl hydroperoxide and the A₃₄₀ is recorded. The rate of decrease in the A₃₄₀ is directly proportional to the GPx activity in the sample.

References

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For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.