CREATIVE BIOMART®
Assay Kit

Cellular Glutathione Peroxidase Assay Kit

Product Information

Cat.No.

Kit-0387

Size

1 kit

Description

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. 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-O-H) 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. 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.

Storage

All reagents should be stored at 2-8 oC and are Stable, if unopened, until the expiration date.

Kit Components

1. NADPH Reagent: ß-nicotinamide-adenine dinucleotide phosphate (reduced), Glutathione, and Glutathione reductase. Lyophilized and when reconstituted with 7.5mL Assay Buffer, each of the 5 vials provide for 20 tests.2. Assay Buffer: pH 7.6, 120 mL3. tert-Butyl Hydroperoxide: In water, 2.0 mL

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Compatible Sample Types

Samples that may be assessed using this assay include:• Purified c-GPx: Purified enzyme can be assayed without any special preparation. However, the enzyme stock should be frozen, preferably at -70 oC at 3000 mU/mL or more, in a buffer containing 1 mM dithiothreitol or 2-mercaptoethanol (or other thiol reducing agent), 5 mM EDTA, and 1 mg/mL protein (for example, bovine IgG) to preserve enzyme activity.• Erythrocyte lysates: Recommended sample size is 0.2-0.5 mg of hemoglobin or protein (from a clarified lysate) (e.g., 70 µL of 7 mg/mL) added to the reaction mixture.• Plasma or Serum: Plasma or serum are not recommended for use as samples. It has been demonstrated recently that serum albumin may have peroxidase activity. If the researcher is interested in measuring the secreted (plasma) form of GPx (pl·GPx), then Oxford Product FR 16 is recommended.• Cell and Tissue Homogenates: GPx activity may be assayed in most tissue or cell extracts. It is recommended that approximately 0.1 mg to 1 mg of protein (e.g., 70 µL of 1.5-15 mg/mL) be added to the assay in preliminary experiments to establish the range of activity expected in the particular tissue.

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