

Nitric Oxide and Cell Stress

Detection of stress-inducing markers (reactive oxygen species (ROS), peroxides, heat shock proteins) as well as the measurement of the levels of detoxifying and stress-reducing molecules (glutathione reductase, glutathione peroxidase, heme oxygenase) is essential for the study of cellular states, cell damage, cell death and damage prevention.

Peroxides generate hydroxyl or peroxy reactive radicals that can interact with DNA, proteins or lipid components of cells, causing cell damage that can lead to cell death. The measurement of peroxides helps determine the level of free radicals present in specific tissues. Measurement of hydrogen peroxide in tissues has been used to study several aspects of free radical damage such as skin aging induced by UV light and the effect of hydrogen peroxide as an inducer of elevated tyrosinase levels in melanoma cells. Hydrogen peroxide has been shown to be a potent mitogen for growth-arrested cultured human aortic smooth muscle cells.

Cellular glutathione peroxidase (EC 1.11.1.9, c-GPx) detoxifies peroxides in cells. Levels of c-GPx are a reflection of the pathogenic state of the cell. There is a marked increase in the level of c-GPx in reticulocytes in diabetic rats and normal levels are restored after administration of insulin. A decrease in the level of GPx has been observed in Favism (a disease associated with extreme hemolytic crisis) and in patients suffering from hairy cell leukemia.

Glutathione reductase (EC 1.6.4.2) (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). GR is essential for maintaining adequate levels of reduced cellular GSH, which serves as an antioxidant. Glutathione is also a substrate for c-GPx and glutathione S-transferases in the detoxification of organic peroxides and the metabolism of xenobiotics.

Heme oxygenase (HO) catalyzes the oxidation of heme to biliverdin, with the release of iron and carbon monoxide. Two forms of heme oxygenase have been identified, heme oxygenase-1 (HO-1, also known as Heat Shock Protein 32) and heme oxygenase-2 (HO-2). HO-1 is induced by several stimuli including heavy metals, heme, endotoxins, inflammatory cytokines, prostaglandins and phorbol esters. HO-1 activity is found in many types of cells and tissues including, liver, spleen and bone marrow. Analysis of HO-1 expression can be used to estimate the oxidative stress response.

New Nitric Oxide and Cell Stress Assay Kits

New Colorimetric Hydrogen Peroxide (H₂O₂) Assay

Prod. No. CS0270

Hydrogen Peroxide (H₂O₂) is a reactive oxygen metabolic by-product linked to asthma, inflammatory arthritis, arteriosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Measurement of H₂O₂ will help to determine how oxidative stress modulates various intracellular pathways.

Assay Platform

The kit is designed to measure low concentrations of H₂O₂ in biological matrices. A color reagent produces a purple color proportional to the concentration of H₂O₂ in the samples or standards. The mechanism of the color reaction probably involves coordinated iron reacting with H₂O₂ and the dye molecule. The color reaction is read at 540-570 nm in the microplate spectrophotometer. Plot absorbance versus standard H₂O₂ concentrations to quantitate H₂O₂ in the samples.

Samples

A wide range of samples may be used and diluted in 50 mM phosphate, pH 6.0 or cell culture media.

Advantages

Simple

- No sample extraction or lysis required
- One step procedure with one reagent

Sensitive

- Detects H₂O₂ at 51.25 ng/mL when diluted in buffer

Quantitative

- Standard curve is run in each assay

Very Fast

- Results available within 30 minutes

Performance Characteristics

Precision

- Intra-assay variability from 3-10%
- Inter-assay variability from 1.7-5.7%

Linearity

- Dilutions of H₂O₂ are run multiple times in the same assay
- Sample standard curve had a slope of 0.9342 with a coefficient of variation of 0.9983

Recovery

- Plasma, urine and serum samples spiked with H₂O₂ and diluted 1:64 gave >90% recovery

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New Methylguanine-DNA Methyltransferase (MGMT) Assay Kit

Prod. No. MD0100

MGMT (O⁶-Methylguanine-DNA methyltransferase) is a ubiquitous DNA repair protein that removes O⁶-alkyl-guanine lesions, primarily O⁶-methyl-guanine, from damaged DNA. It is a major contributor to cellular protection from the mutagenic, carcinogenic and cytotoxic effects of DNA alkylation.

A correlation exists between the occurrence of cancer in various tissues and the lack of the MGMT enzyme. Therefore, MGMT is a target for both over-expression studies aimed to protect hematopoietic stem cells from cancer, and drug discovery studies aimed at identifying inhibitors to be used in conjunction with oncologic alkylating agents.

The MGMT Assay Kit is based on the formation of a new restriction site in a 23-mer ³²P labeled ds-oligonucleotide after removal of the O⁶-methyl-guanine lesion. Cleavage of the new restriction site results in two smaller oligonucleotides that can be resolved on a denaturing PAGE gel.

The kit can be used for:

- Screening for MGMT inhibitors.
- Detection of MGMT activity in cell lines.

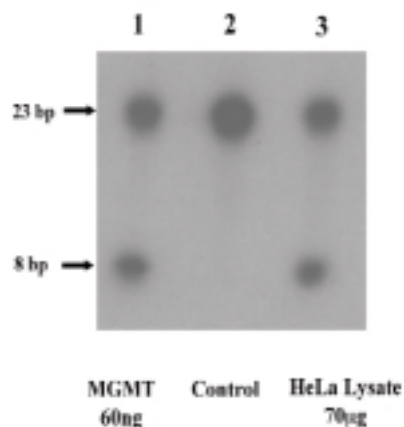
The kit is sufficient for 100-200 tests.

Components

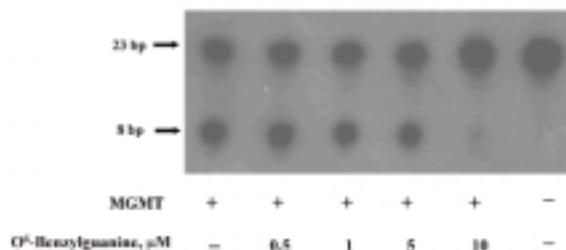
MGMT Substrate O⁶-methylguanine strand, 1 vial
 MGMT Substrate complementary strand, 1 vial
 Reaction buffer 10X, 1.5 mL
 Stop solution, 1 mL
 Methylguanine-DNA methyltransferase (MGMT), 10 µL
 O⁶-Benzylguanine solution, 50 µL
 Spin column-10, 2 each

References

1. Hansen, W.K. and Kelly, M.R., *J. Pharmacol. Exp. Ther.*, **295**, 1-9 (2000).
2. Silber, J.R., et al., *Proc. Natl. Acad. Sci. USA*, **93**, 6941-6946 (1996).
3. Lees, N.P., et al., *Brit. J. Cancer*, **87**, 169-170 (2002).
4. Jansen, M., et al., *Cancer Gene Ther.*, **9**, 737-46 (2002).
5. KUDOS Pharmaceuticals, www.kudospharma.co.uk/r_d/patrin.php



MGMT activity in HeLa cell lysate. MGMT activity in HeLa cell lysate was determined using the MGMT Assay Kit (Prod. No. **MD0100**). The substrate used was a 23 bp oligonucleotide containing an O⁶-methylguanine lesion. Lane 1 - MGMT positive control (60 ng), Lane 2 - Control, Lane 3 - HeLa cell lysate (70 µg). The upper band of the autoradiogram represents the uncleaved substrate (23 bp), while the lower band represents the cleavage product (8 bp).



MGMT activity inhibition by O⁶-benzylguanine. The inhibition of MGMT activity was assayed using the MGMT Assay Kit (Prod. No. **MD0100**). MGMT (50 ng) was incubated with increasing concentrations of O⁶-benzylguanine for 30 min, followed by MGMT activity determination using a 23 bp substrate containing a O⁶-methylguanine lesion. The upper band of the autoradiogram represents the uncleaved substrate (23 bp), while the lower band represents the cleavage product (8 bp). O⁶-benzylguanine at a concentration of 10 mM completely inhibits MGMT activity, while the effect of lower inhibitor concentrations are insignificant.

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Nitric Oxide and Cell Stress

Nitric Oxide and Cell Stress Assay Kits

Glutathione Peroxidase Cellular Activity Assay Kit

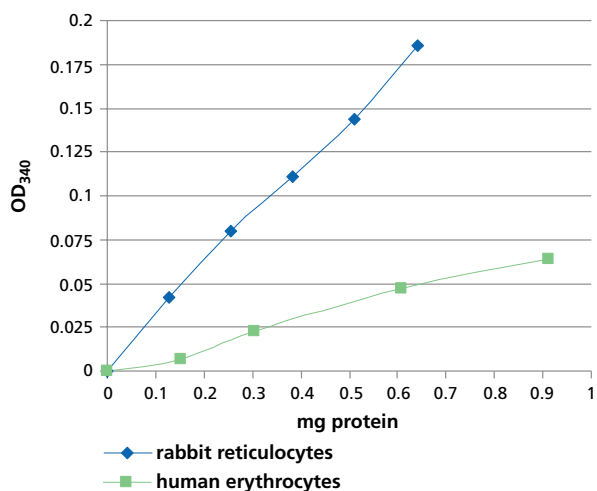
Prod. No. CGP-1

This kit can be used to assay glutathione peroxidase (GPx) in tissue extracts. The assay is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx. GSSG is recycled back to GSH utilizing glutathione reductase and NADPH. The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP is indicative of GPx activity since the enzyme is the rate-limiting factor of the coupled reactions. This kit was tested with rabbit reticulocyte lysate and with GPx enzyme.

Sufficient for 100 tests

Components:

tert-Butyl hydroperoxide, 1 mL
Glutathione peroxidase assay buffer, 120 mL
NADPH assay reagent, 5 vials



Glutathione Peroxidase Assay of Blood Cell Lysates. The activity of cellular glutathione peroxidase in a rabbit reticulocyte lysate and a human erythrocyte lysate was measured using the Glutathione Peroxidase Cellular Activity Assay Kit (Prod. No. CGP-1). Varying amounts of both lysates were assayed for activity after a ten-fold dilution with glutathione peroxidase assay buffer.

Glutathione Reductase Assay Kit

Prod. No. GR-SA

This kit contains reagents for a spectrophotometric assay of glutathione reductase activity either by following the decrease in absorbance at 340 nm caused by the oxidation of NADPH (UV assay) or the increase in absorption at 412 nm caused by the reduction of dithiobis(2-nitrobenzoic acid; DTNB) (colorimetric assay).

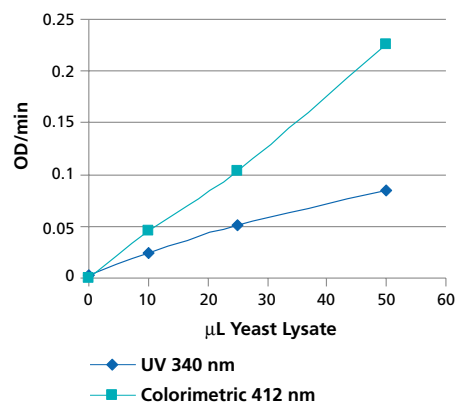
Unit definition: UV assay: One unit will cause the oxidation of 1.0 μ mole of NADPH at 25°C at pH 7.5.

Colorimetric assay: One unit will cause the reduction of 1.0 μ mole of DTNB to TNB at 25°C at pH 7.5.

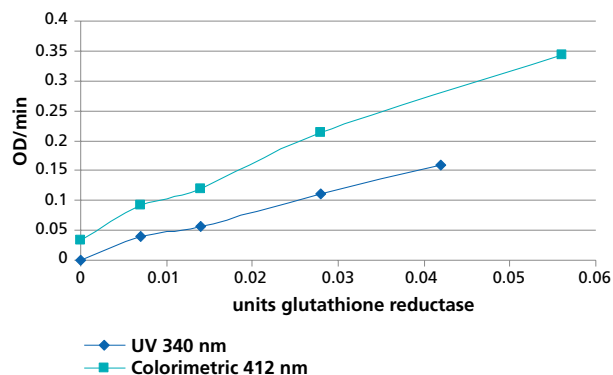
Sufficient for 100 tests

Components

5,5'-Dithiobis(2-nitrobenzoic acid), 50 mg
Glutathione, oxidized, disodium salt, 100 mg
Glutathione reductase assay buffer, 125 mL
Glutathione reductase dilution buffer, 100 mL
Glutathione reductase positive control, 1 unit
 β -Nicotinamide adenine dinucleotide, 5 vials



Assay of Purified Glutathione Reductase. Glutathione reductase was diluted with dilution buffer to 1.4 units/mL. The dilution was assayed with both the UV and colorimetric assays provided with the Glutathione Reductase Assay kit (Prod. No. GR-SA).



Assay of Yeast Lysate. The activity of glutathione reductase in *Saccharomyces cerevisiae* cell lysate (8.8 mg protein/mL) was assayed with both the UV and colorimetric assays provided with the Glutathione Reductase Assay Kit (Prod. No. GR-SA).