EnzyChrom™ Glutathione Reductase Kit (ECGR-100)

Quantitative Colorimetric Kinetic Glutathione Reductase Activity Determination

DESCRIPTION

GLUTATHIONE REDUCTASE (GR) reduces oxidized glutathione (GSSG) to the reduced sulfhydryl form GSH which is an important cellular antioxidant. A high GSH/GSSG ratio is important for protection against oxidative stress. Thus, measurement of GR activity is used as indicator for oxidative stress. BioAssay Systems' non-radioactive, colorimetric GR assay is designed to accurately measure GR activity in biological samples with a method that utilizes Ellman's method in which DTNB reacts with the GSH generated from the reduction of GSSG by the GR in a sample to form a yellow product (TNB^{2-}). The rate of change in the optical density, measured at 412 nm, is directly proportional to GR activity in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.4 to 50 U/L for 20 min reaction. Detection Limit of 0.4 U/L.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

GR activity determination in biological samples (e.g. plasma, serum, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**GDH:**	120 µL
Substrate:	1 mL	**DTNB:**	60 µL
Cosubstrate:	1 mL	**Calibrator:**	1.5 mL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Calibrator: Transfer 100 µL of Calbrator and 100 µL Assay Buffer to separate wells in a 96 well plate.

Reagent Preparation: equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all samples by mixing, for each 96-well assay: 8 µL Substrate, 8 µL Cosubstrate, 1 µL GDH, 0.5 µL DTNB and 70 µL Assay Buffer.

Reaction Preparation:

1. Transfer 20 µL of each sample into separate wells and add 80 µL WR to each sample well. Tap plate briefly to mix.
2. Incubate plate at desired temperature for 10 min and read OD_{412nm} (OD_{10}), and again after 30 min (OD_{30}) on a plate reader.

CALCULATION

Subtract the OD_{10} from OD_{30} for each sample to compute the ΔOD_S. GR activity can then be calculated as follows:

$$\text{GR Activity} = \frac{\Delta OD_S}{2 \bullet \varepsilon_{TNB} \bullet l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \bullet \text{Sample Vol (µL)}} \times n$$

$$= \frac{440}{t\,\text{(min)}} \times \frac{\Delta OD_S}{(OD_{CAL} - OD_{Buffer})} \times n \quad \textit{(U/L)}$$

where ε_{TNB} is the molar absorption coefficient of TNB and 2 is the number of moles of TNB generated for each mole of GSSG converted by GR. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{Buffer} are OD_{412nm} (OD_o) values of the Calibrator and Assay Buffer. t is the reaction time (20 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

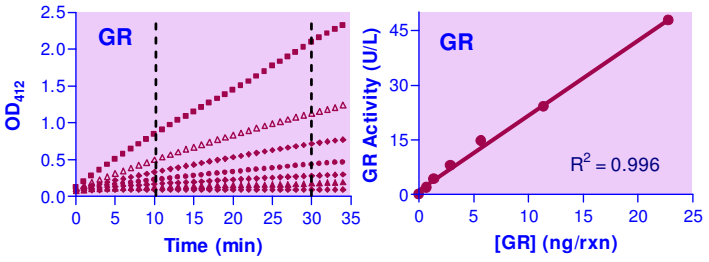
Unit definition: 1 Unit (U) of GR will catalyze the conversion of 1 µmole of GSSG to 2 µmole GSH per min at pH 7.6.

Note: If sample GR activity exceeds 50 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.

GR Titration Curves



Raw Kinetic Data *GR Activity (20 min, 25°C)*

LITERATURE

1. Delides, A et. al. (1976). An optimized semi-automatic rate method for serum glutathione reductase activity and its application to patients with malignant disease. J. Clin. Path. 29: 73-7.
2. Smith, IK et. Al. (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 175: 408-13.
3. Cribb, AR et. Al. (1989) Use of a microplate reader in an assay of glutathione reductase using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 183: 195-6.