Glutathione Reductase (GR) Assay Kit (Fluorometric)

LS-K277-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio's 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 (TNB2-). The rate of change in the optical density, measured at 412 nm, is directly proportional to GR activity in the sample.

Key Features

- Sensitive and accurate. Linear detection range in 96-well plate: 0.1 to 50 U/L for colorimetric assays and 0.01 to 2 U/L for fluorometric assays run at 25°C for 30 min.

Applications

- 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.

Components

Component	K277-100
	100 Tests
Assay Buffer	12 mL
Substrate	1 mL
Cosubstrate	1 mL
GDH	120 µL
DTNB	60 µL
Calibrator	1.5 mL

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

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 OD412nm (OD10), and again after 30 min (OD30) on a plate reader.

Calculations

Subtract the OD10 from OD30 for each sample to compute the ΔODS. GR activity can then be calculated as follows:

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

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

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.

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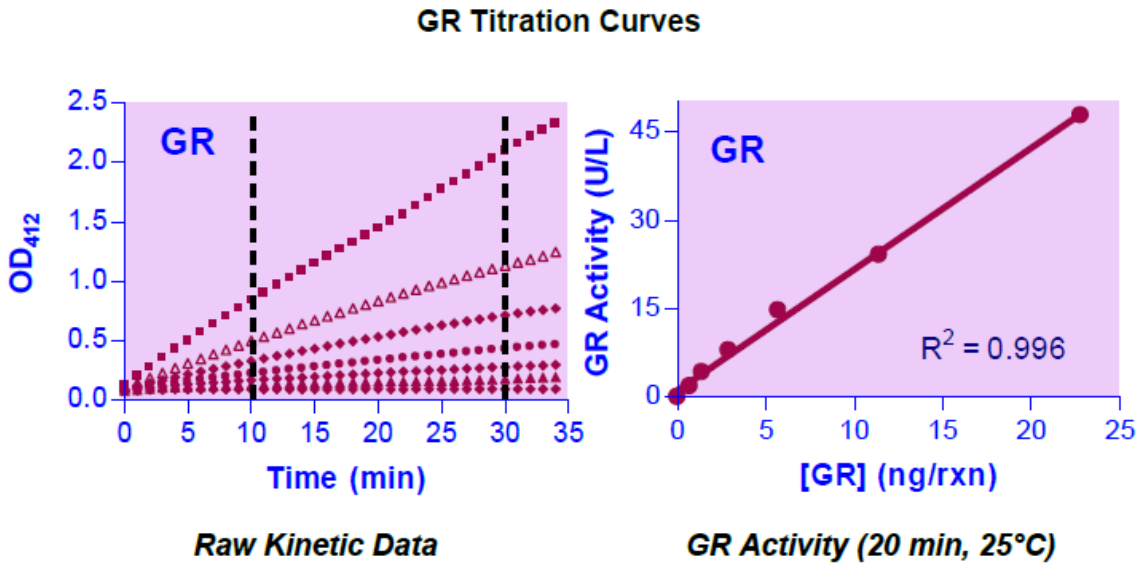
Sample Data



Version: V.08.09.2018