ab83461

Glutathione Reductase (GR) Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Glutathione Reductase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

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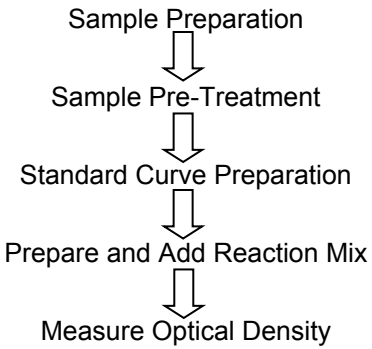
1. Overview

Glutathione Reductase (GR, EC 1.8.1.7) catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which plays an important role in the GSH redox cycle that maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress.

Abcam's Glutathione Reductase Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric assay for measuring GR activity in biological samples. In the assay, GR reduces GSSG to GSH, which reacts with 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) to generate TNB^{2-} (yellow color, λ_{max} = 405 nm). The assay can detect 0.1-40 mU/ml GR in various samples.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
GR Assay Buffer	100 mL
3 % H_2O_2	1 mL
Catalase (Lyophilized)	1 vial
TNB Standard (2.5 μmol)	1 vial
DTNB (Lyophilized)	1 vial
NADPH-GNERAT™ (Lyophilized)	2 vials
GSSG (Lyophilized)	1 vial
GR Positive Control (10 mU; Lyophilized)	1 vial

* Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep samples, NADPH-GNERAT™ solution and GR standard on ice during the assay. Read the entire protocol before performing the assay.

CATALASE: Dissolve with 1 mL Assay Buffer. The Catalase solution is stable for 1 week at 4°C and 1 month at -20°C.

TNB STANDARD: Dissolve with 0.5 mL Assay Buffer to generate 5 mM TNB Standard. The TNB standard solution is stable for 1 week at 4 °C and 1 month at -20°C.

DTNB SOLUTION: Dissolve with 0.45 mL Assay Buffer, sufficient for 200 assays. The DTNB solution is stable for 2 weeks at 4°C and 1 month at -20°C.

NADPH-GNERAT™: Dissolve with 0.22 mL Assay Buffer; sufficient for 100 assays. The solution is stable for 10 hours at 4°C and 2 weeks at -20°C.

GSSG: Dissolve with 1.3 mL Assay Buffer, sufficient for 200 assays. The GSSG solution is stable for 2 weeks at 4°C and 2 months at -20°C.

GR POSITIVE CONTROL: Dissolve lyophilized GR into 100 µL Assay Buffer, aliquot into vials, store at -20°C. It is stable for 1 day at 4°C and 1 month at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Homogenize 0.1 gram tissues on ice in 0.5-1.0 mL cold assay buffer, or 1 x 10^6 cells, or 0.2 mL Erythrocytes on ice in 0.1-0.2 mL cold assay buffer. Centrifuge at 10,000 x g for 15 min at 4°C. Collect the supernatant for assay and store on ice, serum can be tested directly. Store at -80°C.

2. Sample Pre-Treatment:

Samples should be treated to destroy GSH before the assay. Take 100 µL sample, add 5 µL 3% H_2O_2, mix and incubate at 25°C for 5 min. Then add 5 µL of Catalase, mix and incubate at 25°C for another 5 min. Add 2-50 µL of the pre-treated samples into a 96-well plate, bring the volume to 50 µL with Assay Buffer.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range. As a guideline:

- Cell lysates: use a volume that corresponds to 1-5x10^5 cells per well,
- Tissue lysates: 10-50 µg of extracted protein per well
- Biological fluids: undiluted

Use 10 µL/well Positive Control (optional) and adjust to 50 µL with Assay Buffer.

3. TNB Standard Curve:

Add 0, 2, 4, 6, 8, 10 µL of the TNB Standard into 96-well plate in duplicate to generate 0, 10, 20, 30, 40, 50 nmol/well standard. Bring the final volume to 100 µL with Assay Buffer.

Optional. To control for background levels in samples with colour,, measure OD_{405nm} at T_0 before adding reaction mix. OD_{405nm} values above sample buffer should be subtracted from the result values from readings A_1 and A_2.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

GR Assay Buffer	40 µL
DTNB solution	2 µL
NADPH-GNERAT™ solution	2 µL
GSSG solution	6 µL

Add 50 µL of the Reaction Mix to each test samples. Mix well.

Note. Wells with standard do not need Reaction Mix.

5. Immediately measure OD_{405nm} at T_1 (reading A_1). Incubate the reaction at 25°C for 10 min (or incubate longer time if the GR activity is low), protect from light, measure OD_{405nm} again at T_2 (reading A_2).

$$\mathbf{\Delta A_{405nm} = A_2 - A_1.}$$

Note:

It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics, and ensure A_1 and A_2 are in the reaction linear range.

5. Data Analysis

Plot the TNB standard Curve. Apply the ΔA_{405nm} to the TNB standard curve to get ΔB nmol of TNB.

$$\text{GR Activity} = \frac{\Delta B}{(T_2 - T_1) \times 0.9 \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

ΔB is the TNB amount from TNB standard Curve (in nmol).

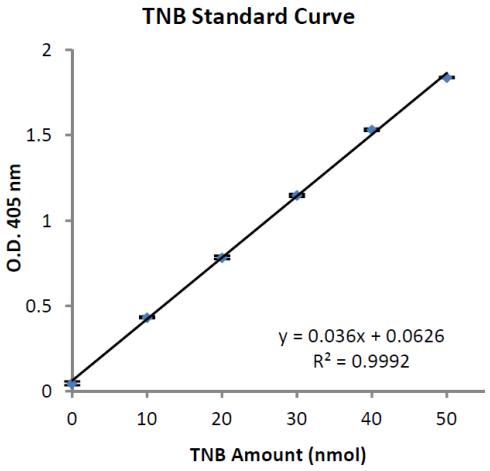
T_1 is the time of the first reading (A_1) (in min).

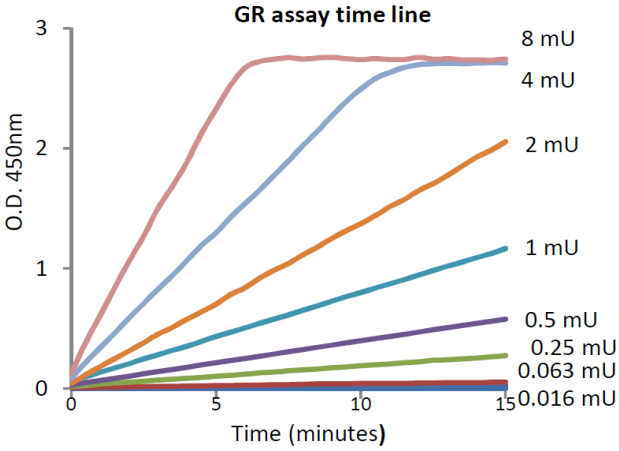
T_2 is the time of the second reading (A_2) (in min).

V is the pretreated sample volume added into the reaction well (in mL).

0.9 is the sample volume change factor during sample pre-treatment procedure.

Unit Definition: One unit is defined as the amount of enzyme that generates 1.0 μmol of TNB per minute at 25°C. The oxidation of 1 mole of NADPH to $NADP^+$ will generate 2 mol TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.


TNB Standard Curve
O.D. 405 nm
TNB Amount (nmol)
y = 0.036x + 0.0626
R² = 0.9992
0
0.5
1
1.5
2
0
10
20
30
40
50


GR assay time line
O.D. 450nm
Time (minutes)
8 mU
4 mU
2 mU
1 mU
0.5 mU
0.25 mU
0.063 mU
0.016 mU
0
1
2
3
0
5
10
15

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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