

**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.

# Table of Contents

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1. Overview	2
2. Protocol Summary	2
3. Components and Storage	3
4. Assay Protocol	6
5. Data Analysis	8
6. Troubleshooting	10

# 1. Overview

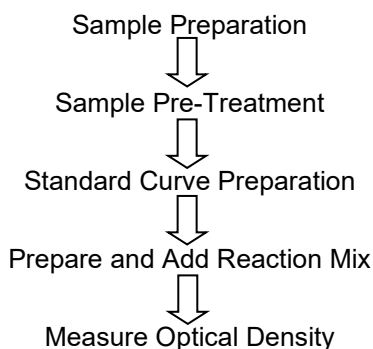
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Glutathione Reductase (GR, EC 1.8.1.7) catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which plays an important role in the GSH redox cycle that maintains adequate levels of reduced GSH. A high GSH/GSSG 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<sup>2-</sup> (yellow color,  $\lambda_{\max} = 405 \text{ nm}$ ). The assay can detect 0.1-40 mU/ml GR in various samples.

# 2. Protocol Summary

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### 3. Components and Storage

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\*All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Briefly centrifuge all small vials prior to opening. Keep samples, NADPH Generating Mix solution and GR standard on ice during the assay. Read the entire protocol before performing the assay.

#### A. Kit Components

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Item	Quantity	Storage Temperature (before prep)
Assay Buffer 41	100 mL	-20°C
Hemin Substrate	1 mL	-20°C or 4°C
Catalase	1 vial	-20°C
TNB Standard	1 vial	-20°C
DTNB	1 vial	-20°C
NADPH Generating Mix	4 vials	-20°C
GSSG	1 vial	-20°C
GR Positive Control	1 vial	-20°C

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PLEASE NOTE: Assay Buffer 41 was previously labelled as Assay Buffer XLI and GR Assay Buffer, and Hemin Substrate as Substrate V and 3 % H<sub>2</sub>O<sub>2</sub>. The composition has not changed

HEMIN SUBSTRATE: Store at 4°C after reconstitution.

ASSAY BUFFER 41: Warm to room temperature before use.

CATALASE: Dissolve with 1 mL Assay Buffer 41. 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 41 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: Dissolve with 0.45 mL Assay Buffer 41, sufficient for 200 assays. To dissolve completely, heat to 37°C for 2 minutes, vortex for 10 seconds and sonicate for up to 5 minutes as necessary. Pipette entire volume into a pipette tip to ensure completely dissolved. The DTNB solution is stable for 2 weeks at 4°C and 1 month at -20°C.

NADPH Generating Mix: Dissolve with 0.22 mL Assay Buffer 41; sufficient for 100 assays. The solution is stable for 4 hours at 4°C and 24 hours at -20°C.

GSSG: Dissolve with 1.3 mL Assay Buffer 41, 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 200  $\mu$ L Assay Buffer 41, aliquot into vials, store at  $-20^{\circ}\text{C}$ . It is stable for 1 day at  $4^{\circ}\text{C}$  and 1 month at  $-20^{\circ}\text{C}$ .

### **Additional Materials Required**

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

## 4. Assay Protocol

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### 1. Sample Preparation:

Homogenize 0.1 gram tissues on ice in 0.5-1.0 mL cold Assay Buffer 41, or  $1 \times 10^6$  cells, or 0.2 mL Erythrocytes on ice in 0.1-0.2 mL cold Assay Buffer 41. Centrifuge at  $10,000 \times g$  for 15 min at  $4^\circ\text{C}$ . Collect the supernatant for assay and store on ice, serum can be tested directly. Store at  $-80^\circ\text{C}$ .

### 2. Sample Pre-Treatment:

Samples should be treated to destroy GSH before the assay. Take 100  $\mu\text{L}$  sample, add 5  $\mu\text{L}$  Hemin Substrate, mix and incubate at  $25^\circ\text{C}$  for 5 min. Then add 5  $\mu\text{L}$  of Catalase, mix and incubate at  $25^\circ\text{C}$  for another 5 min. Add 2-50  $\mu\text{L}$  of the pre-treated samples into a 96-well plate, bring the volume to 50  $\mu\text{L}$  with Assay Buffer 41.

*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  $1-5 \times 10^5$  cells per well,
- Tissue lysates: 10-50  $\mu\text{g}$  of extracted protein per well
- Biological fluids: undiluted

Use 10  $\mu\text{L}$ /well Positive Control (optional) and adjust to 50  $\mu\text{L}$  with Assay Buffer 41.

### 3. TNB Standard Curve:

Add 0, 2, 4, 6, 8, 10  $\mu\text{L}$  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  $\mu\text{L}$  with Assay Buffer 41.

Optional. To control for background levels in samples with colour, measure  $\text{OD}_{405\text{nm}}$  at  $T_0$  before adding reaction mix.  $\text{OD}_{405\text{nm}}$  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  $\mu\text{L}$  Reaction Mix:

Assay Buffer 41	40 $\mu\text{L}$
DTNB	2 $\mu\text{L}$
NADPH Generating Mix	2 $\mu\text{L}$
GSSG	6 $\mu\text{L}$

Add 50  $\mu\text{L}$  of the Reaction Mix to each test samples. Mix well.

**Note. Wells with standard do not need Reaction Mix.**

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

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

**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

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Plot the TNB standard Curve. Apply the  $\Delta A_{405\text{nm}}$  to the TNB standard curve to get  $\Delta B$  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:

$\Delta B$  is the TNB amount from TNB standard Curve (in nmol).

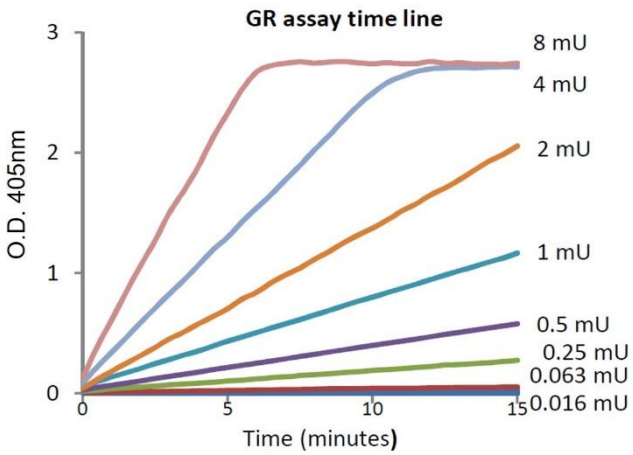
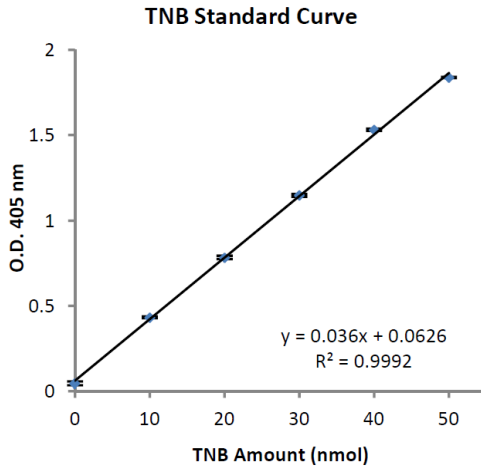
$T_1$  is the time of the first reading ( $A_1$ ) (in min).

$T_2$  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  $\mu\text{mol}$  of TNB per minute at 25°C. The oxidation of 1 mole of NADPH to NADP<sup>+</sup> will generate 2 moles of GSH from 1 mole of GSSG, finally producing 2 moles of TNB from 1 mole of DTNB. Therefore, 1 TNB unit equals 0.5 NADP unit.



## 6. Troubleshooting

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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 <b>10kDa spin column (ab93349)</b>
	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)

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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

**Technical Support**

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