Product Information

Glutathione Reductase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2173-200 200 assays	Cap Color	Part Number
GR Assay Buffer	100 ml	NM	K2173-C-1
3 % H_2O_2	1 ml	Orange	K2173-C-2
Catalase (lyophilized)	1 vial	Clear	K2173-C-3
TNB Standard (lyophilized)	1 vial	Brown	K2173-C-4
DTNB (lyophilized)	1 vial	Red	K2173-C-5
NADPH-GNERAT™ (lyophilized)	2 vials	Blue	K2173-C-6
GSSG (lyophilized)	1 vial	Yellow	K2173-C-7
GR Positive Control (10 mU; lyophilized)	1 vial	Green	K2173-C-8

II. Introduction:

Glutathione Reductase (GR) mediates the GSH redox cycle that keep the normal levels of reduced GSH. It catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). High GSH/GSSG ratio protects cell from oxidative damage. The Glutathione Reductase Activity Colorimetric Assay Kit provides an easy and sensitive way of detecting GR activity in biological samples. GR reduces GSSG to GSH, which reacts with 5, 5′-Dithiobis (2-nitrobenzoic acid) (DTNB) to generate TNB2- (yellow color, λmax = 405 nm). The detection limit is 0.1 – 40 mU/ml.

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Catalase: Dissolve lyophilized catalase 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 in 0.5 ml of assay buffer to generate 5 mM TNB Standard. The TNB standard solution is stable for at least 2 months at -20°C.

DTNB Solution: Dissolve DTNB 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 one vial 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 GSSG 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.

Ensure that the Assay Buffer is at room temperature before use. Keep samples NADPH-GNERAT™ solution and GR standard on ice during the assay.

V. Glutathione Reductase Activity Assay:

1. Sample Preparations: Homogenize 0.1 gram tissues, or 1 x 10^6 Cells, or 0.1 ml Erythrocytes on ice in 4 volumes of 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 Pretreatment: 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 pretreated 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. 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.

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. Measure OD 405 nm at T1 (reading A1). Incubate the reaction at 25°C for 10 min (or incubate longer time if the GR activity is low), protect from light, measure OD 405 nm again at T2 (reading A2). ΔA405 nm = A2 – A1.

Note: It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics, and ensure A1 and A2 in the reaction linear range.

5. Calculation: Plot the TNB standard Curve. Apply the ΔA405nm to the TNB standard curve to get ΔB nmol of TNB (TNB amount generated between T1 and T2 in the reaction wells).

$$\text{GR Activity} = \Delta B / [(T2-T1) \times 0.9 \times V] \times \text{Sample dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$$

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

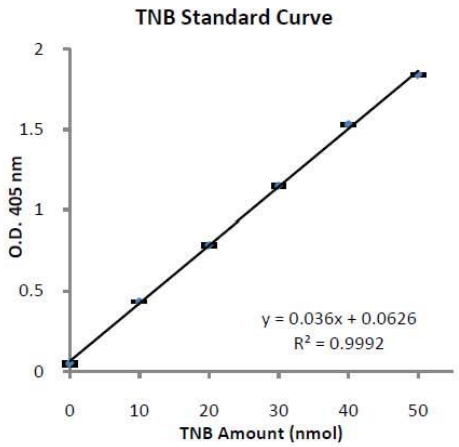
T1 is the time of the first reading (A1) (in min).

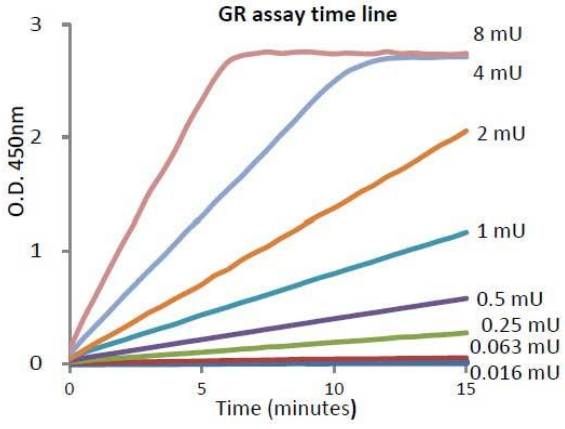
T2 is the time of the second reading (A2) (in min).

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 mole TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the reaction mix • Air bubbles formed in well • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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