

Glutathione Reductase Assay Kit

(Catalog #K761-200; 200 reactions; Store kit at -20°C)

I. Introduction:

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. BioVision'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 TNB²⁻ (yellow color, λ_{max} = 405 nm). The assay can detect 0.1 – 40 mU/ml GR in various samples.

II. Kit Contents:

Components	K761-200	Cap Code	Part No.
GR Assay Buffer	100 ml	NM	K761-200-1
3% H ₂ O ₂	1 ml	Orange	K761-200-2
Catalase	1 vial	Clear	K761-200-3
TNB Standard (5 mM)	1 vial	Brown	K761-200-4
DTNB	1 vial	Red	K761-200-5
NADPH-GNERAT™	2 vials	Blue	K761-200-6
GSSG	1 vial	Yellow	K761-200-7
GR Positive Control (10 mU)	1 vial	Green	K761-200-8

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 lyophilized TNB standard 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 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 50 µl GR Solution 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 10⁶ Cells, or 0.2 ml Erythrocytes on ice in 0.5-1.0 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. Keep samples at -80°C for storage.

2. Sample Pretreatment:

Samples should be treated to destroy GSH before the assay. Take 100 µl sample, add 5 µl 3% H₂O₂, 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.

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:

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

Add 50 µl of the Reaction Mix to each test samples. Mix well. Measure O.D.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 O.D.405 nm again at T2 (reading A2). $\Delta A_{405\text{nm}} = A2 - A1$.

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 $\Delta A_{405\text{nm}}$ to the TNB standard curve to get ΔB nmol of TNB (TNB amount generated between T1 and T2 in the reaction wells).

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

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

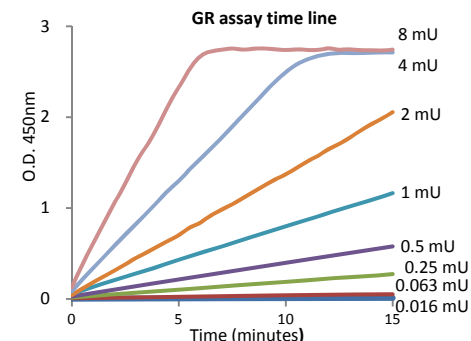
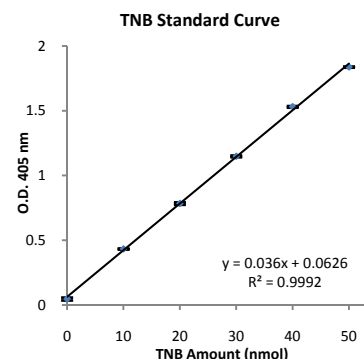
T1 is the time of the first reading (A1) (in min).

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.

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.



VI. Related Products:

- Colorimetric Glutathione Detection Kit
- Glutathione Kit (GSH, GSSG and Total)
- GST Colorimetric Assay Kit
- Acid Phosphatase Assay Kit
- Phosphate Fluorescence Assay Kit
- NAD/NADH Quantification Kit
- Pyruvate Assay Kit
- Ammonia Assay Kit
- Glucose Assay Kit
- Ethanol Assay Kit

- ApoGSH Glutathione Detection Kit
- GST Fluorometric Assay Kit
- Triglyceride Assay Kit
- ADP/ATP Ratio Assay Kit
- Phosphate Colorimetric Assay Kit
- NADP/NADPH Quantitation Kit
- Lactate Assay Kit/ II
- Glutamate Assay Kit
- Fatty Acid Assay Kit
- Uric Acid Assay Kit