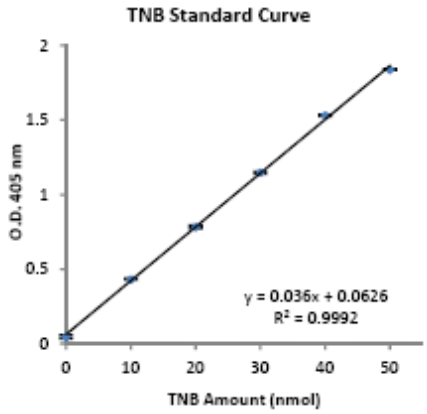
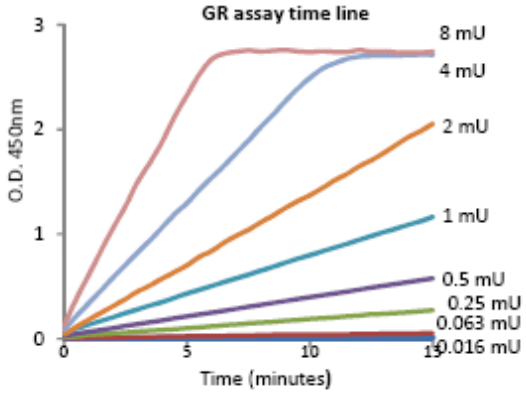
Colorimetric Glutathione Reductase Activity Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K761
Description	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. PromoKine's Colorimetric Glutathione Reductase Activity 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, λmax = 405 nm). The assay can detect 0.1 – 40 mU/ml GR in various samples.
Quantity	200 assays
Kit Components	Components / Quantity / Cap Code GR Assay Buffer / 100 ml / NM 3% H2O2 / 1 ml / Orange Catalase / 1 vial / Clear TNB Standard (2.5 µmol) / 1 vial / Brown DTNB / 1 vial / Red NADPH-GNERAT™ / 2 vials / Blue GSSG / 1 vial / Yellow GR Positive Control (10 mU) / 1 vial / Green
Applications / Assay Protocol	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. A. 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. B. Glutathione Reductase Activity Assay: 1. Sample Preparations: Homogenize 0.1 gram tissues, or 1 x 10^6 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 minutes 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% H_2O_2, mix and incubate at 25°C for 5 minutes. Then add 5 µl of catalase, mix and incubate at 25°C for another 5 minutes. 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: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl 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 minutes (or incubate longer time if the GR activity is low), protect from light, measure O.D.405 nm again at T2 (reading A2). ΔA405nm = 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). $$\text{GR Activity} = \frac{\Delta B}{(T2 - T1) \times 0.9 \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$$ 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.  
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40