CheKine™ Reduced Glutathione (GSH) Colorimetric Assay Kit

Item NO.	Product Name
KTB1600	CheKine™ Reduced Glutathione (GSH) Colorimetric Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the red blood cell, reduced glutathione (GSH) is the key to maintain hemoglobin in reduced state and protect cells from oxidative damage. GSH is the most important antioxidant sulfhydryl compound in cells, which plays an important role in oxidation resistance, protein sulfhydryl protection and amino acid transport across membrane. The ratio between reduced and oxidized glutathione (GSH / GSSG) is the main indicator of the cell's redox state. Therefore, measuring the content of GSH and GSSG and the ratio of GSH / GSSG in cells can reflect the redox state of cells.

Assay principle

DTNB reacts with reduced glutathione to form a yellow product. The optical density measured at 412 nm, can directly reflect glutathione concentration in the sample.

Performance

Detection range: 200-2ug/mL Sensitivity: 2ug/mL

Storage/Stability

Storage at -20°C and protected from light. Stable for at least 12 months at recommended temperature from the date of shipment.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures. Please refer to the Safety Data Sheet for hazards and safe practices information.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if used separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity		Storage conditions
	48T	96T	
Extraction Buffer	50 mL	100 mL	4°C
Assay Buffer	10 mL	20 mL	4°C
Chromogen	4 mL	8 mL	4°C, protected from light
Standard	1 vial	1 vial	4°C

Other materials required, Not Supplied

- Standard microplate reader, capable of measuring absorbance at 412 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (for tissue samples)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips frequently. Also, use separate reservoirs for each reagent.
- Ensure all equipment, reagents and solutions at the appropriate temperature before starting the assay.
- If the sample value is higher than the maximum standard value, please further dilute the sample.

ASSAY PROTOCOL

Reagent preparation

Extraction Buffer：Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC.
Assay Buffer：Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC.
Chromogen: Ready to use as supplied. Store at 4ºC protected from light.
Standard：Ready to use as supplied. Store at 4ºC protected from light.

Standard preparation

Notes: Always prepare fresh standards per use; Diluted standard solution is unstable and must be used within 4 hours.

Prepare 1mg/mL GSH Standard by dissolve 1 mg standard with 1 mL deionized water. Make a 1:10 dilution of the Extraction Buffer solution with deionized water in a clean plastic tube by diluting 200 µL Extraction Buffer into 1800 µL deionized water. Prepare 200ug/mL of GSH Standard by diluting 200 µL 1mg/mL Standard into 800 µL diluted Extraction Buffer. Using 200µg/mL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

	Volume of 200µg/mL Standard	Diluted Extraction Buffer	Concentration (µg/mL)
Std.1	100µL	0 µL	200
Std.2	50 µL	50 µL	100
Std.3	25 µL	75 µL	50
Std.4	12.5µL	87.5µL	25
Std.5	5 µL	95 µL	10
Std.6	2.5 µL	97.5 µL	5
Std.7	1µL	99 µL	2
Blank	0	100 µL	0

Sample preparation

Note: The sample can be kept at -80°C for 10 days. If not do experiments immediately.

Tissue samples: Wash tissue with cold PBS to remove blood as much as possible. Homogenize tissue at 1 mL/0.1 g in Extraction Buffer. Centrifuge at 8000 rpm for 10 minutes at 4°C. Use supernatant for GSH assay.
Plasma samples: Collect plasma using an anticoagulant. Centrifuge at 600 g for 10 minutes at 4°C. Collect supernatant within 30 minutes and add equal volume of Extraction Buffer. Centrifuge at 8000 g for 10 minutes at 4°C. Use supernatant for GSH assay.
Blood cells samples: Collect blood using an anticoagulant. Centrifuge at 600 g for 10 minutes at 4°C. Discard the upper plasma, then wash the pellet with triple volume of cold PBS 3 times (use PBS resuspend blood cells, centrifuge at 600 g for 10 minutes at 4°C). Add equal volume of Extraction Buffer, then mix and stand at 4°C for 10 minutes. Centrifuge at 8000 g for 10 minutes at 4°C. Use supernatant for GSH assay.
Cell (adherent or suspension) Samples: Collect appropriate number of cells for each assay (initial recommendation= 1-2 x 10^6 cells/assay). Wash cells with cold PBS twice (Resuspend cells with PBS, centrifuge at 600 g for 10 minutes at 4°C). Resuspend cells in triple volume of cells pellet Extraction Buffer, repeated freeze-thaw cycles 2-3 times (can be frozen in liquid nitrogen, dissolved in 37°C water bath). Centrifuge at 8000 g for 10 minutes at 4°C. Use supernatant for GSH assay.

Assay procedure

1. Prepare all the reagents, diluted standards and sample as directed in the previous sections.
2. Add 20 µL of diluted standards and sample per well.
3. Add 140 µL of Assay Buffer to all well, then add 40 µL of Chromogen to all well (multi-channel pipettor is recommended). Tap plate to mix.
4. Incubate for 2 minutes at room temperature, protected from light.
5. Measure the optical density of each well with microplate reader at 412 nm.

DATA ANALYSIS

Calculation of results

1. Subtract blank OD from the standard OD values and plot the OD against standard concentrations.
2. Determine the slope using linear regression fitting. The GSH concentration of Sample is calculated as

$$[\mathrm{GSH}] = \frac{\mathrm{OD}_{\mathrm{SAMPLE}} - \mathrm{OD}_{\mathrm{BLANK}}}{\mathrm{Slope}} \times \mathrm{n} \quad (\mu g/ml)$$

Note: If the OD_{SAMPLE} values are higher than the OD value of the 200μg/mL standard, dilute sample with deionized water and repeat this assay. Multiply the results with the dilution factor: n.

Typical data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay.