

Glutathione Detection Kit I

Colorimetric detection of glutathione changes in apoptosis and other pathological processes

Instruction Manual

Catalog Number: PK-CA577-K261

Description
 Glutathione (GSH) is the major intracellular low-molecular-weight thiol that plays a critical role in the cellular defense against oxidative stress in mammalian cells. The PromoKine Colorimetric Glutathione Detection Kit provides a convenient, colorimetric method for analyzing either total glutathione or the reduced form of glutathione only using a microtiter plate reader. The assay is based on the glutathione recycling system by DTNB and glutathione reductase (Fig. 1). DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and GSSG. Since 2-nitro-5-thiobenzoic acid is a yellow colored product, GSH concentration can be determined by measuring absorbance at 412 nm. GSH can be regenerated from GSSG by glutathione reductase, and reacts with DTNB again to produce more 2-nitro-5-thiobenzoic acid. Therefore, the recycling system dramatically improves the sensitivity of total glutathione detection. The kit includes the 5-Sulfosalicylic acid (SSA) for the removal of proteins from samples and for the protection of GSH oxidation and γ -glutamyl transpeptidase reaction. The kit can quantify glutathione from 1-100 ng/well in a 200 μ l reaction. For detecting lower glutathione concentrations, such as in blood samples, increasing reaction time will generate stronger signal. The kit can also specifically detect the reduced form of glutathione (GSH) by omitting the glutathione reductase from the reaction mixture. The sensitivity for detecting the reduced form of glutathione (without recycling system) is 100 times lower than detecting the total glutathione.

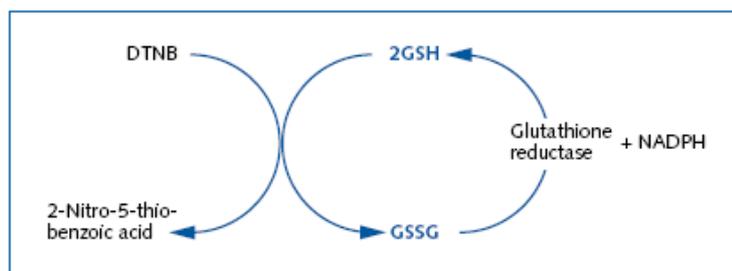


Fig. 1: Principle of the Colorimetric Glutathione Detection Kit.

Quantity: 100 assays

Kit Components	Components	Quantity	Color Code
	Glutathione Reaction Buffer	100 ml	NM
	Glutathione Substrate (DTNB)	2 vials	Red
	NADPH Generating Mix (lyophilized)	2 vials	Blue
	Glutathione Reductase (lyophilized)	2 x 25 μ l	Green
	Sulfosalicylic Acid (SSA, 1 gram)	1 bottle	WM
	GSH Standard (lyophilized, MW 307)	2 x 1 mg	Yellow

Applications / Assay Protocol

I. Sample Preparation:

A. Cell Sample Preparation (0.5-1 x 10⁶ cells/assay)

1. Treat cells by desired method. Concurrently incubate a control culture without treatment.
2. Collect cells by centrifugation at 700 x g for 5 minutes at 4°C. Remove supernatant.
3. Resuspend cell pellet in 0.5 ml ice-cold PBS. Transfer into a 1.5 ml microcentrifuge tube, and centrifuge at 700 x g for 5 minutes at 4°C. Remove supernatant.
4. Lyse cells in 80 μ l ice-cold Glutathione Reaction Buffer. Incubate on ice for 10 minutes.
5. Add 20 μ l of 5% SSA (see below for SSA preparation), mix well and centrifuge at 8,000 x g for 10 minutes. Transfer supernatant to a fresh tube and use it for glutathione assay.

B. Tissue Sample Preparation (100 mg)

1. Homogenize the tissue in 0.4 ml of Glutathione Buffer.
2. Add 100 μ l of 5% SSA (see below for SSA preparation), mix well, and centrifuge at 8,000 x g for 10 minutes.
3. Transfer supernatant to a fresh tube and use it for glutathione assay.

C. Plasma Sample Preparation

1. Centrifuge an anticoagulant treated blood at 1,000 x g for 10 minutes at 4°C.
2. Transfer the top plasma layer to a new tube and add 1/4 vol of 5% SSA. Mix well.
3. Centrifuge at 8,000 x g for 10 minutes at 4°C.
4. Transfer supernatant to a new tube, and use it for the glutathione assay.

D. Erythrocyte Sample Preparation

1. Centrifuge an anticoagulant treated blood at 1,000 x g for 10 minutes at 4°C.
2. Discard the supernatant and the white buffy layer.
3. Lyse the erythrocytes with 4 vol. of Glutathione Reaction Buffer. Keep on ice for 10 minutes.
4. Add 1 vol. 5% SSA, mix well, and centrifuge at 8,000 x g for 10 minutes. Transfer supernatant to a fresh tube and use it for glutathione assay.

Note: Erythrocytes can be isolated from the remaining sample solution after the plasma sample isolation.

II. Preparation of Solutions & Storage Conditions:

Substrate: Add 1 ml of Glutathione Reaction Buffer to 1 vial of substrate and dissolve it completely. Store the remaining solution at -20°C, stable for 2 months.

NADPH Generating Mix: Add 1 ml of Glutathione Reaction Buffer to 1 vial of the NADPH mix. Store the solution at -20°C, stable for 2 months.

Glutathione Reductase: Add 1 ml of Glutathione Reaction Buffer to 1 vial of the enzyme (25 µl) and mix. Use up the solution within 1 day.

SSA: Add 19 ml of dH₂O to make 5% solution and then dilute 5 ml of the solution with Glutathione Reaction Buffer to make 1% solution. Store at 4°C, stable for 6 months.

GSH Standard: Add 1 ml of 1% SSA to the GSH standard vial to generate 1 µg/µl GSH standard solution. Store at -20°C, stable for 2 months.

III. Preparation of Solutions for Standard Curve:

To generate standard curve for detecting the reduced form of glutathione only, add 50, 40, 30, 20, 10, and 0 µl of the 1 µg/µl GSH standard into each labeled microcentrifuge tube, add 1% SSA to make up for a total volume of 100 µl/tube.

To generate standard curve for detecting the total glutathione, dilute the 1 µg/µl glutathione solution into 10 ng/µl with 1% SSA. Add 50, 40, 30, 20, 10, and 0 µl of the 10 ng/µl GSH standard into each labeled microcentrifuge tube, add 1% SSA to make up for a total volume of 100 µl/tube.

IV. Glutathione Assay Protocol:

1. Prepare enough Reaction Mix for the standard and samples to be assayed in a 96-well plate (not provided). Each well should contain:

- 20 µl NADPH Generating Mix
- 20 µl Glutathione Reductase*
- 120 µl Glutathione Reaction Buffer

*For detecting the reduced form of glutathione only, omit Glutathione Reductase. Use 20 µl of the Glutathione Reaction Buffer to replace the 20 µl of Glutathione Reductase.

2. Mix well. Add 160 µl of the Reaction Mix to each well and incubate at room temperature for 10 minutes to generate NADPH.
3. Add 20 µl of either the GSH standard solutions or the sample solution. Incubate the plate at room temperature for 5-10 minutes.

Note: We recommend to make several dilutions of your sample using the 1% SSA to make sure the readings are within the range of the standard calibration curve.

4. Add 20 µl of Substrate solution, and incubate at room temperature for 5-10 minutes, or longer if the samples contain low levels of glutathione.

Notes: a) Since the reaction starts immediately after the addition of substrate, use a multichannel pipette or repeating pipette is recommended to avoid the reaction time lag among wells.

b) You can read samples immediately and at various times following addition of the substrate solution for kinetic studies.

4. Read the absorbance at 405 nm or 415 nm using a microplate reader.

	<p>5. Determine concentrations of GSH in the sample solutions using the standard glutathione calibration curve.</p> <p>Notes:</p> <p>A. Using reduced form Glutathione Standard Curve for detecting reduced form of glutathione. Using total Glutathione Standard Curve for detecting total glutathione. There are about 10 to 100 fold difference in detection sensitivity between detecting reduced form glutathione and total glutathione (see procedure step IV for preparation of standard curve).</p> <p>B. The colorimetric reaction is stable and the O.D. increases linearly over 30 minutes for total glutathione detection.</p> <p>VI. Calculation of Total Glutathione</p> <p>Pseudo-end point method: Total Glutathione = (O.D.[sample] - O.D.[blank])</p> <p>Kinetic method: Total Glutathione = (Slope [sample] - Slope [blank])/Slope</p> <p>V. Reagent Interference</p> <p>Reducing agents such as ascorbic acid, β-mercaptoethanol, dithiothreitol (DTT) and cysteine, or thiol reactive compounds such as maleimide compounds, interfere with the glutathione assay and therefore should be avoided during the sample preparation.</p> <p>When detecting the reduced form of glutathione, protein thiols can generate significant background signal. In such cases, it is necessary to completely remove proteins from samples. We suggest using Amicon Centrifugal Spin column with 5K molecular weight cut off filter to remove proteins. Then the reduced glutathione can be easily detected from spin through samples.</p>
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.

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