Quantification of total glutathione

Total Glutathione Quantification Kit

Product Code: T419

Glutathione (GSH) is the most abundant thiol compound in animal tissues, plant tissues, bacteria, and yeast. GSH has many different roles, including protection against reactive oxygen species and the maintenance of protein thiol groups. During these processes, GSH is converted into its oxidized form, glutathione disulfide (GSSG). Since GSSG is then enzymatically reduced by glutathione reductase, GSH is the dominant form in organisms.
DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)), known as Ellman's Reagent, was developed to detect thiol compounds. In 1985, Dr. M. E. Anderson suggested that the glutathione recycling system involving DTNB and glutathione reductase could be used as a highly sensitive glutathione detection method. DTNB and GSH react to generate 2-nitro-5-thiobenzoic acid and GSSG (Fig. 1). Since 2-nitro-5-thiobenzoic acid is yellow, the GSH concentration in a sample solution can be determined by O.D. measurement at 412 nm absorbance (Fig. 2). GSH is regenerated from GSSG by glutathione reductase and will again react with DTNB to produce 2-nitro-5-thiobenzoic acid. This recycling reaction improves the sensitivity of total glutathione detection.

Contents of the Kit

Substrate(DTNB)	2 vials
Enzyme Solution	50 µl x 1
Coenzyme(lyophilized)	2 vials
Standard GSH (lyophilized)	1 vial
Buffer Solution	50 ml x 1

Required Equipment & Materials

Microplate Reader (405 or 415 nm filter)
96-well microplate
20-200 µl multi-channel pipettes
Incubator (37°C)
5-sulfosalicylic acid(SSA)

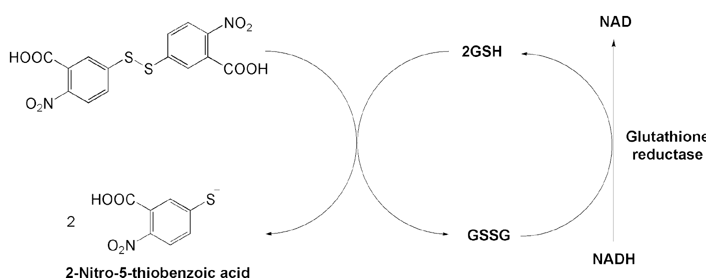

Fig.1 Principal of total glutathione detection

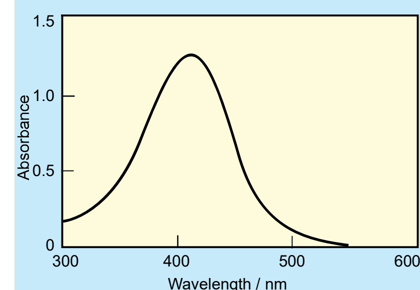

Fig. 2 Absorption spectrum of 5-Mercapto-2-nitrobenzoic acid

1. Preparation of Sample Solutions

► Cell(adhesive cell: 5 x 10^5 cells, leukocyte cell: 1 x 10^6 cells)

1. Collect cells by centrifugation at 200 g for 10 minutes at 4°C. Discard the supernatant.
2. Wash the cells with 300 µl PBS and centrifuge at 200 g for 10 minutes at 4°C. Discard the supernatant.
3. Add 80 µl 10 mM HCl, and lyse the cells by freezing and thawing twice.
4. Add 20 µl 5% SSA and centrifuge at 8,000 g for 10 minutes.
5. Transfer the supernatant to a new tube, and use it for the assay. If the final concentration of SSA is over 1%, add ddH_2O to reduce the concentration of SSA from 0.5 to 1%.

► Tissue(100 mg)

1. Homogenize the tissue in 0.5-1.0 ml 5% SSA.
2. Centrifuge the homogenized tissue sample at 8,000 g for 10 minutes.
3. Transfer the supernatant to a new tube and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay.

► Plasma

1. Centrifuge anticoagulant-treated blood at 1,000 g for 10 minutes at 4°C.
2. Transfer the top plasma layer to a new tube and add 5% SSA equivalent to half of the volume of the plasma.
3. Centrifuge at 8,000 g for 10 minutes at 4°C
4. Transfer the supernatant to a new tube, and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay.

► Erythrocyte

1. Centrifuge anticoagulant-treated blood at 1,000 g for 10 minutes at 4°C.
2. Discard the supernatant and the white buffy layer.
3. Lyse the erythrocytes with 5% SSA equivalent to 4 times the volume of the erythrocytes.
4. Centrifuge at 8,000 g for 10 minutes at 4°C.
5. Transfer the supernatant to a new tube, and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay. Erythrocytes can be isolated from the remaining sample solution after the plasma sample isolation.

*Preparation of 5% 5-Sulfosalicylic Acid (SSA) Solution
Note: SSA is not included in this kit.
1. Dissolve 1 g SSA in 19 ml water.
2. Store the solution at 4°C (stable for 6 months at 4°C).

2. Preparations of Solutions

► Substrate working solution

Add 1.2 ml of Buffer Solution to one vial of Substrate, and dissolve.
Store the solution at -20°C (stable for 2 months).

► Enzyme working solution

Mix Enzyme Solution using pipette. Take out 20 µl of Enzyme Solution, and mix it with 4 ml of Buffer Solution.
Store the solution at 4°C (stable for 2 months).

► Coenzyme working solution

Add 1.2 ml of ddH_2O to the Coenzyme vial and dissolve. The Coenzyme vial is decompressed. Use a syringe to add ddH_2O, then open the vial.
Store the solution at -20°C(stable for 2 months).

► GSH standard solution

Add 2 ml of 0.5% SSA to Standard GSH vial, and dissolve to prepare 200 µM of GSH standard solution. The Standard GSH vial is decompressed. Use a syringe to add 0.5% SSA, then open the vial.
Store the solution at -20°C(stable for 2 months).
Dilute 100 µl of 200 µM GSH standard solution by serial dilution with 100 ul of 0.5% SSA in plastic tubes as indicated Fig. 4.

3. General Protocol (refer to Fig. 3)

1. Add 20 µl of Enzyme working solution, 20 µl of Coenzyme working solution and 120 µl of Buffer Solution to each well.
2. Incubate the plate at 37°C for 5 minutes.
3. Add 20 µl of GSH standard solution and 20 µl of sample solution to each well.
4. Incubate the plate at 37°C for 10 minutes.
5. Add 20 µl of Substrate working solution, and incubate the plate at room temperature for 10 minutes.
6. Read the absorbance at 405 nm or 415 nm using a microplate reader.
7. Determin concentrations of GSH in the sample solutions using a calibration curve. Since the colorimetric reaction is stable and the O. D. increases linearly over 30 min. A time course of the colorimetric reaction is shown Fig. 5. Typical calibration curves prepared using the pseudo-endpoint method is indicated in Fig. 6.

4. Calculation of total glutathione (GSH and GSSG) concentration

Determine the total glutathione concentration[a] in a sample solution using the following equations.

► pseudo-end point method

Total glutathione (GSH+GSSG)=(O.D.$_{sample}$ - O.D.$_{blank}$)/slope[b]

► kinetic method

Total glutathione (GSH+GSSG)=(Slope[c]$_{sample}$ - Slope[c]$_{blank}$)/slope[b]

a) Since the values obtained by these equations are the amount of total glutathione in treated sample solutions, further calculations are necessary if the actual concentrations of glutathione in samples need to be determined.
b) slope of the calibration curve
c) slope of the kinetic reaction

5. Interference

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. Therefore, SH compounds, reducing agents and SH reactive materials should be avoided during the sample preparation.

6. Notes

- The kit is stable for 6 months at 0 to 5°C.
- Use the reagents in the kit after the reagents temperature are equilibrated to the room temperature.
- Triplicate measurements per sample is recommended to obtain accurate data.
- Since the colorimetric reaction starts immediately after the addition of Substrate working solution to a well, use a multichannel pipette to avoid the reaction time lag of each well.
- If the concentration range of total glutathione in a sample is unknown, prepare multi-diluted sample solutions.
- This kit is not for GSSG/GSH ratio determination. For GSSG/GSH ratio determination, please refer to GSSG/GSH Quantification Kit.

7. References

1. G. L. Ellman, Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959;**82**:70-77.
2. O. W. Griffith, Determination of Glutathione and Glutathione Disulfide Using Glutathione Reductase and 2-Vinylpyridine. *Anal Biochem*. 1980;**106**:207-212.
3. M. E. Anderson, Determination of Glutathione and Glutathione Disulfide in Biological Samples. *Methods Enzymol*. 1985;**113**:548-555.
4. M. A. Baker, *et al*., Microtiter Plate Assay for the Measurement of Glutathione and Glutathione Disulfide in Large Numbers of Biological Samples. *Anal Biochem*. 1990;**190**:360-365.

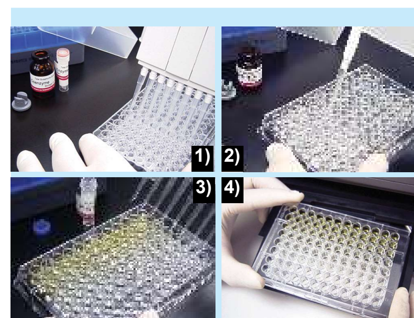

Fig. 3 Assay procedure

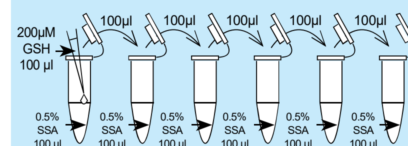

Fig. 4 Serial dilution process

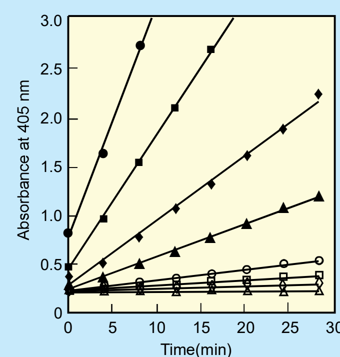

Fig. 5 Time-dependent Abs. increase on eight diferent samples

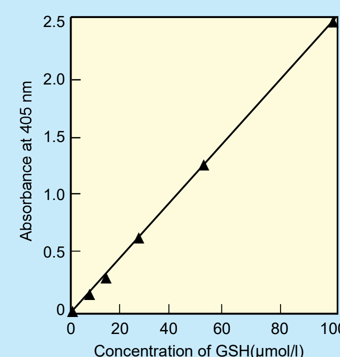

Fig. 6 Calibration curve prepared using pseudo-endpoint method(10 min incubation at room temperature)

8. Recent Publications

Title	Reference
Copper Chaperone-Dependent and -Independent Activation of Three Copper-Zinc Superoxide Dismutase Homologs Localized in Different Cellular Compartments in Arabidopsis	C. Huang, *et al. Plant Physiology.* 2012; **158**: 737 - 746
IRE1α activation protects mice against acetaminophen-induced hepatotoxicity	K. Yeon, *et al., J. Exp. Med.* 2012; **209**: 307 - 318
The Nitric Oxide Prodrug JS-K Is Effective against Non–Small-Cell Lung Cancer Cells In Vitro and In Vivo: Involvement of Reactive Oxygen Species	A.. Maciag, *et al., J. Pharmacol. Exp. Ther.*, 2011; **336**: 313 - 320
Mutations in PNKD causing paroxysmal dyskinesia alters protein cleavage and stability	Y. Shen, *et al., Hum. Mol. Genet*., 2011; **20**: 2322 - 2332
Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species	S. Suzuki, *et al., PNAS*, 2010; **107**: 7461 - 7466
Oxidative Stress and Sodium Methyldithiocarbamate–Induced Modulation of the Macrophage Response to Lipopolysaccharide In Vivo	S. Pruett, *et al., Toxicol. Sci.* 2009; **109**: 237 - 246
Nrf2 Enhances Cell Proliferation and Resistance to Anticancer Drugs in Human Lung Cancer	S. Homma, *et al., Clin. Cancer Res.* 2009; **15**: 3423 - 3432
Inhibition of hepatic Niemann-Pick C1-like 1 improves hepatic insulin resistance	K. Irie, *et al., Journal of Dental Research*. 2008; **87**: 456 - 460
3-Morpholinopropyl isothiocyanate is a novel synthetic isothiocyanate that strongly induces the antioxidant response element-dependent Nrf2-mediated detoxifying/antioxidant enzymes in vitro and in vivo	Y. Keum, *et al., Carcinogenesis.* 2008; **29**: 594 - 599
Cells Deficient in the FANC/BRCA Pathway Are Hypersensitive to Plasma Levels of Formaldehyde	J. Ridpath, *et al., Cancer Res.* 2007; **67**: 11117 - 11122

9. FAQ

►Do I have to dilute the sample solution prior to the assay?

If you do not know the total glutathione level of your sample, multiple dilutions may be necessary. If the total glutathione level of your sample is less than 100 μM, no dilution is necessary.

►What interferes with the assay?

Reducing agents (such as ascorbic acid, beta-mercaptoethanol, dithiothreitol, and cysteine) and thiol reactive compounds (such as maleimides) interfere with the glutathione assay. Therefore, reducing agents and thiol reactive compounds should be avoided during the sample preparation.