Distinguish Measurement of Glutathione

GSSG/GSH Quantification Kit

Product Code: G257

Glutathione (γ-L-glutamyl-L-cysteinylglycine) is a tripeptide present in the body, and it is involved in antioxidation, drug metabolism, and other as enzyme substrate of glutathione peroxidase, glutathione *S*-transferase, and thiol transferase, etc. Glutathione is usually present as reduced form (GSH), but GSH is converted into its oxidized form (GSSG) by stimulation such as oxidative stress. Therefore, the ratio of GSH and GSSG has been noted as index of oxidative stress.
The GSSG/GSH Quantification kit contains Masking Reagent of GSH. The GSH can be deactivated in the sample by adding the Masking Reagent. Therefore, only the GSSG is detected by measuring the absorption (λmax = 412nm) of DTNB (5,5'-dithiobis (2-nitrobenzoic acid) using the enzymatic recycling system. Also, GSH can be determined the quantity by subtracting GSSG from the total amount of glutathione.
The kit can be limited to quantify GSH/GSSG concentration from 0.5 µmol/l to 50 µmol/l and GSSG concentration from 0.5 µmol/l to 25 µmol/l.

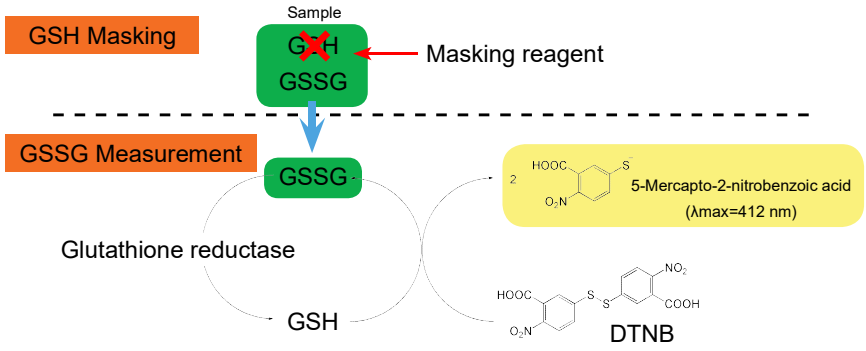

Fig.1 Principal of GSSG/GSH detection

1. General Protocol

► Preparation of Sample Solution
Please refer to Total Glutathione Quantification, page 5.

► Determination of GSSG concentration

1. Add 4 µl of Masking Solution to sample solution and 200 µl of GSSG standard solution diluted with 0.5% SSA respectively, then transfer 40 µl of the solution to each well.
2. Add 120 µl of Buffer Solution to each well and incubate for 1 hour at 37°C.
3. Add 20 µl of Substrate working solution to each well, then add 20 µl of Coenzyme working solution and Enzyme working solution to each well respectively.
4. Incubate for 10 minutes at 37°C and read the absorbance at 405 or 415 nm using a microplate reader.
5. Determine concentrations of GSSG in the sample solution using a GSSG calibration curve (Fig. 2).

► Determination of total glutathione concentration

1. Add sample solution and 40 µl of GSH standard solution diluted with 0.5% SSA to each well.
2. Add 120 µl of Buffer solution to each well and incubate for 1 hour at 37°C.
3. Add 20 µl of Substrate working solution to each well, then add 20 µl of Coenzyme working solution and Enzyme working solution to each well respectively.
4. Incubate for 10 minutes at 37°C and read the absorbance at 405 or 415 nm using a microplate reader.
5. Determine concentrations of total glutathione in the sample solution using a GSH calibration curve(Fig. 3).

► Calculating the concentration of GSH
GSH(conc.) = total Glutathione(conc.) - 2 x GSSG(conc.)

2. Recent Publications

Title	Reference
Diurnal Variation of cadmium-induced mortality in mice	N. Miura, and T. Hasegawa, *et al., J. Toxicol. Sci.* 2012; **37**: 191

Contents of the Kit

Enzyme Solution	50 µl x 1
Coenzyme	2 vials
Buffer Solution	60 ml x 1
Substrate (DTNB)	4 vials
Standard GSH	1 vial
Standard GSSG	1 vial
Masking Reagent	20 µl 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)
Ethanol

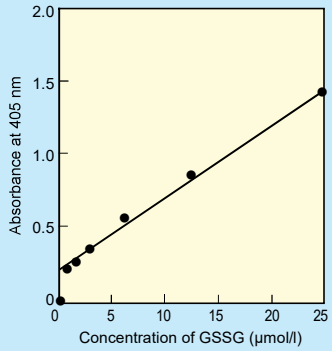

Fig. 2 Determination of the concentration of GSSG

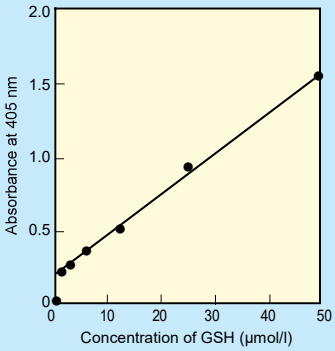

Fig. 3 Determination of the concentration of total glutathione