Glutathione (GSH) Reduced Assay Kit

Cat No. GSH-M-50
Size: 50 Reactions
Store at 4°C under dark conditions

MOLEQULE-ON®

Components	Quantity
Standard	10 mg
Reagent I	50 ml
Reagent II	50 ml
Reagent III	15 ml

Description

Glutathione (GSH) is a natural three peptide composed of glutamic acid (Glu), cysteine (Cys) and glycine (Gly), which is a compound containing sulfhydryl group (SH), it is an important antioxidant in plants, animals, fungi, and some bacteria and archaea. Glutathione reaction with 5,5'-dithiobis-2-nitrobenoic acid (DTNB) to form a yellow product. It has the maximum absorption at 412nm.

Reagents and Equipment Required but Not Provided:

Analytical balance, micro-homogenizer (2ml), refrigerated centrifuge, water-bath, Micropipette, Spectrophotometer, 1ml cuvette.

Sample Preparation

1. Tissue sample

Wash the fresh tissues twice with PBS, then add 0.1g washed tissue sample into homogenizer (Before using the homogenizer, wash with Solution I pre-cooled on ice). Add 1ml Solution I (the proportion of tissue and reagents can be kept constant), fully grind the tissue on ice (grinding under liquid nitrogen will have a better grinding effect). Centrifuge at 8000 rpm for 10 min at 4℃. Keep supernatant at 4 ℃ for test (The supernatant can be stored at -80℃ for 10 days.)

2. Blood sample

a. Plasma： Centrifuge the sample at 600 g for 10 min at 4℃. Transfer the upper plasma into another tube and add same volume of Solution I, centrifuge at 8000 g for 10 min at 4℃. Place supernatant at 4℃ for test. (The Supernatant can be stored at -80℃ for 10 days).

b. Blood cell： Centrifuge the sample at 600 g for 10 min at 4℃. Discard the upper plasma, wash the settled blood cells with 3 times volumes of PBS (mix blood cell with PBS, centrifuge at 600 g for 10 min). Repeat the washing step two more times. In the washed blood cells, add equal volume of Solution I. Centrifuge at 8000 g for 10 min. Keep supernatant at 4℃ for test (The supernatant can be stored at -80℃ for 10 days).

3. Cell sample

Harvested cell should not be less than 10^8. Wash with PBS twice (mix cell with PBS, centrifuge at 600 g for 10 min)，wash with 3 times volumes of PBS for 3 times. Perform repeated freezing and thawing 2-3 times (suggest freezing in liquid nitrogen, and thawing at 37 ℃ water bath). Centrifuge at 8000 g for 10min. Place supernatant at 4 ℃ for test (The supernatant can be stored at -80℃ for 10 days).

Protocol

1. Prewarm Spectrophotometer for 30 min, adjust the wavelength to 412 nm, set the counter to zero with distilled water.
2. Preheat Solution Ⅱ in water bath: 37℃ (mammal cell) , 25℃ (other species).
3. Blank tube detection: Add 100 μl distilled water, 700 μl Solution Ⅱ and 200 μl Solution Ⅲ into 1 ml cuvette. Fully mixed for 2 min then determine absorbance A1 at 412nm.
4. Standard curve: 1 mg Standard dissolved with 1 ml distilled water (1mg/ml). Dilute Solution I tenfold, take appropriate solution to prepare standard solution with concentration of 200μg/ml, 100μg/ml, 50μg/ml, 25μg/ml, 12.5μg/ml. Add 100 μl Standard Solution, 700 μl Solution Ⅱ, 200 μl Solution Ⅲ into 1.5 ml centrifuge tube. Fully mixed and place for 2 min, detect the absorbance at 412nm, the values obtained subtract A1 as abscissa, according to the absorbance (x) and concentration (y, g/ml) to make the standard curve.
5. Sample determination: Add 100 μl sample, 700 μl Solution Ⅱ, 200 μl Solution Ⅲ into 1 ml cuvette. Fully mixed and place for 2 min, detect absorbance as A2 at 412nm, △A= A2- A1.

Calculations

According to the standard curve, sample △A into the formula (x), calculate the sample concentration of Y (g/ml).

1. Protein concentration

GSH（μg / mg protein）= y*Vrv/V s/Cpr = y*10/Cpr

2. Sample weight

GSH（μg /g）= y*Vrv/(V s/Vsv*W) = y*10 /W

3. Cell amount

GSH（μg /104 cell）= y*Vrv/(V s/Vsv*n) = y*10 /n

4. Solution volume

GSH（μg / mL）= y*Vrv/Vs = y*10

n: Cell amount
Vrv：Total reaction volume, 1ml；
Vsv：Total supernatant volume, 1 ml；
V s：The volume of supernatant was added into the reaction system, 100μl=0.1 ml；
W：Sample weight, g;
Cpr：Supernatant protein concentration, mg/ml.

Notes

1. The sample needs to be treated completely. If the test cannot be completed temporarily, it can be stored at -80℃.
2. The standard solution should be prepared just before use.
3. If the content of GSH in the sample is uncertain, dilute the sample to several gradients for test.
4. Solution I contained protein precipitant, the supernatant could not be used for protein concentration determination.