



ISO 13485:2016 ISO 9001:2015

Ver.250101

Reduced Glutathione (GSH) Assay Kit

BC4401-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Glutathione is a natural tripeptide composed of glutamic acid (Glu), cysteine (Cys) and glycine (Gly). It is a kind of compound containing sulfhydryl group (-SH), which widely exists in animal tissue, plant tissue, microorganism and yeast. Glutathione can react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce 2-nitro-5-mercaptobenzoic acid and glutathione disulfide (GSSG). 2-nitro-5-mercaptobenzoic acid is a yellow product with the maximum absorption at 412 nm.

Kit components

Reagent	Volume	Storage
Reagent I	60 mL × 1	2-8°C
Reagent II	50 mL × 1	2-8°C
Reagent III	15 mL × 1	2-8°C, in dark
Standard	Powder × 1	2-8°C, in dark
Note: Dissolve the entire contents in 1mL distilled water. prepare aliquots of 500μL and store at -20°C.		

Reagents and Equipment Required but Not Provided

Analytical balance, mortar/homogenizer, low temperature centrifuge, water bath, adjustable pipette, spectrophotometer, 1 mL glass cuvette and distilled water.

Sample preparation

1. Tissue sample

Wash fresh tissues with PBS for twice, then add 0.1 g of sample into homogenizer (the homogenizer has been rinsed with reagent I and placed on ice before use). Add 1 mL reagent I (the proportion of tissue and reagents can be kept constant), fully grinding on ice (using liquid nitrogen will have a better grinding effect). Centrifuge at 8000 ×g for 10 minutes at 4°C, take the supernatant and place it at 4°C for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80°C for 3 days.)

2. Blood sample

Plasma: Sample is centrifuged at 600 ×g for 10 minutes at 4°C. Absorbing the upper plasma into another tube with adding the same volume reagent I. Centrifuge at 8000 ×g for 10 minutes at 4°C, take the supernatant and place it at 4°C for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80°C for 3 days.)

Blood cell: Sample is centrifuged at 600 ×g for 10 minutes at 4°C. Discarding the upper plasma, wash with three times volume of PBS for 3 times (re-suspend blood cell with PBS, centrifuge at 600×g for 10 minutes), add equal volume of reagent I. After mixing, it is placed at 4°C for 10 minutes. Centrifuge at 8000 ×g for 10 minutes, take the supernatant and place it at 4°C for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80°C for 3 days.)

3. Cell sample

Harvesting cell should not less than 10^6 , then wash it with PBS for twice (re-suspend cell with PBS, centrifuge at $600 \times g$ for 10 minutes), The volume of reagent I added is three times the volume of cell precipitation to re-suspend the cells. Repeated freezing and thawing 2-3 times (It is suggested that frozen in liquid nitrogen, dissolved in 37°C water bath) or ultrasonic in ice bath (200w, ultrasound 3second, interval 10second, repeat 30 times). Centrifuge at $8000 \times g$ for 10 minutes, take the supernatant and place it at 4°C for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80°C for 10 days.)

Procedure

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 412 nm, set zero with distilled water.
2. Preparation of standards: aspirate 10mg/mL standard solution and dilute it with distilled water to 200 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$.
3. Operation table: Add the following reagents to the 1.5mL EP tube respectively.

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	100	-	-
Standard	-	100	-
Distilled water	-	-	100
Reagent II	700	700	700

4. Centrifuge at 3000 rpm for 10 minutes
5. Transfer 500 μL supernatant to a new tube.
6. Add 200 μL Reagent III and mix thoroughly and keep at room temperature for 2 minutes.
7. Measure absorbance at 412nm.

$$\text{Absorbance of Standard} = A_S$$

$$\text{Absorbance of Test} = A_T$$

$$\text{Absorbance of Blank} = A_B$$

$$\Delta A_T = A_T - A_B$$

$$\Delta A_S = A_S - A_B.$$

Calculation

According to the concentration of the standard tube (x , $\mu\text{g/mL}$) and the absorbance ΔA_S (y , ΔA_S), a standard curve was established. According to the standard curve, ΔA (y , ΔA) was brought into the formula to calculate the sample concentration (x , $\mu\text{g/mL}$).

1. Protein concentration

$$\begin{aligned}\text{GSH } (\mu\text{g} / \text{mg prot}) &= x \times V_{RV} \div (V_{RV} \times C_{pr}) \\ &= x \div C_{pr}\end{aligned}$$

2. Sample weight

$$\begin{aligned}\text{GSH } (\mu\text{g} / \text{g weight}) &= x \times V_{RV} \div (V_{RV} \div V_{sv} \times W) \\ &= x \div W\end{aligned}$$

3. Cell amount

$$\begin{aligned}\text{GSH } (\mu\text{g}/10^6 \text{ cell}) &= x \times V_{RV} \div (V_{RV} \div V_{SV} \times N) \\ &= x \div N\end{aligned}$$

4. Solution volume

$$\text{GSH } (\mu\text{g}/\text{mL}) = 2x$$

N: Cell amount, count by 10^6

V_{SV} : Total supernatant volume, 1 mL

V_{RV} : Supernatant volume added into the reaction system, 100 μL =0.1 mL

W: Sample weight, g

Cpr: Supernatant protein concentration, mg/mL

2: The volume of plasma (blood cells) is diluted by one time.

Note:

1. The sample needs to be homogenized completely. If the test cannot be completed temporarily, it can be stored at -80°C for 3 days.
2. If the GSH content in the sample is uncertain, Dilute the sample for several gradients before test.
3. Because Reagent I contains protein precipitant, the supernatant cannot be used for protein concentration determination. If the protein content needs to be determined, prepare a new homogenate with PBS.
4. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample volume or dilute the sample before measurement.