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Ver.240823

## 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
Standard	Powder × 1	2-8°C
<b>Note:</b> 10 mg of reduced glutathione (GSH) reagent can be stored at 2-8°C for 6 weeks.		

## 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  $37^\circ\text{C}$  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  $4^\circ\text{C}$  for test. (If the test cannot be completed temporarily, the supernatant can be stored at  $-80^\circ\text{C}$  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 ( $\mu\text{L}$ )	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	100	-	-
Standard	-	100	-
Distilled water	-	-	100
Reagent II	700	700	700
Reagent III	200	200	200

After mixing and stewing at room temperature for 2 minutes, the absorbance at 412 nm of the test tube ,standard tube and blank tube were recorded as  $A_T$ ,  $A_S$  and  $A_B$ , respectively,  
 $\Delta A = A_T - A_B$  and  $\Delta A_S = A_S - A_B$ . The standard curve and blank tube should be done only 1-2 times

#### Calculation

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

##### 1. Protein concentration

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

##### 2. Sample weight

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

### 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  $\mu\text{L}$ =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  $-80^{\circ}\text{C}$  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, take another tissue.
4. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample volume or dilute the sample before measurement.