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Ver.250501

## **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 412nm.

## Kit components

Reagent	Volume	Storage
Extraction Solution	60mL × 1	2-8°C
Reagent I	30mL × 1	2-8°C
Reagent II	40mL × 1	2-8°C
Reagent III	Powder × 1	2-8°C, in dark
Standard	Powder × 1	2-8°C, in dark

**Reagent III working solution:** Dissolve the entire contents of Reagent III in 25mL of Reagent I. Mix thoroughly until fully dissolved. Working solution Stable for 2 weeks at 2–8°C and for 2 months at –20°C. Prepare aliquots before freezing to avoid multiple freeze-thaw cycles.

**Standard:** Add 1mL distilled water directly into the tube. Gently invert the tube multiple times to dissolve the contents completely. Prepare aliquots of 500μL and store at -20°C.

Notes:

- Avoid repeated freeze-thaw cycles by storing in aliquots.

## 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 Extraction Solution and placed on ice before use). Add 1 mL Extraction Solution (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 Extraction Solution. 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 Extraction Solution. 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 Extraction Solution 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 30times).Centrifuge at  $8000 \times g$  for 10minutes, 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 10days.)

### Procedure

- Preheat spectrophotometer for 30 minutes, adjust the wavelength to 412 nm, set zero with distilled water.
- 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 $\mu\text{g/mL}$ .
- 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

- Centrifuge at 3000 rpm for 10 minutes
- Transfer 500 $\mu\text{L}$  supernatant to a new tube.
- Add 200 $\mu\text{L}$  Reagent III and mix thoroughly and keep at room temperature for 2 minutes.
- 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  $\Delta A_s$  ( $y, \Delta A_s$ ), 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

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

#### 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

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

N : Cell amount, count by  $10^6$

V<sub>SV</sub> : Total supernatant volume, 1 mL

V<sub>RV</sub> : Supernatant volume added into the reaction system, 100  $\mu$ 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°C for 3days.
2. If the GSH content in the sample is uncertain, Dilute the sample for several gradients before test.
3. Because Extraction Solution 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.