EZMeta™ Glutathione Oxidized (GSSG) Colorimetric Assay Kit

Cat #: D-AKC1610

Size: 96T

Storage: Stored at -20℃ for 12 months, protected from light

Product Information

Detection range: 1-20 μM

Sensitivity: 1 μM

Applicable samples: Serum, Plasma, Animal/Plant Tissues, Blood cells, Cells, Bacteria

Assay Principle

Glutathione has two forms: reduced form (GSH) and oxidized form (GSSG). GSSG also known as Dithioglutathione, is formed by the oxidation of two molecules of glutathione. GSSG will be reduced to GSH by glutathione reductase (GR), so most of it exists in the reduced form in the body. Determination of intracellular GSH and GSSG content and GSH/GSSG ratio can well reflect the redox state of cells, and is also one of the main indicators of glutathione redox cycle. EZMeta™ Glutathione Oxidized (GSSG) Colorimetric Assay Kit provides a simple, convenient and rapid method for the detection of GSSG content, which is suitable for serum, plasma, animal and plant tissues, blood cells, cells, bacteria, etc. sample. The principle is that reduced Glutathione can react with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) to generate 2-nitro-5-mercaptobenzoic acid, which has a maximum light at a wavelength of 412 nm. Absorption, inhibit the original reduced glutathione in the sample by 2-vinylpyridine, and then use GR to reduce GSSG to GSH, thereby determining the content of oxidized glutathione.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Extraction Buffer	100 mL	4°C
Inhibitor	120 µL	-20°C, protected from light
Assay Buffer	20 mL	4°C
GR	12 µL	4°C, protected from light
GR Cofactor	2	-20°C, protected from light
Chromogen	2	4°C, protected from light
Standard	1	4°C, protected from light

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Refrigerated centrifuge, water bath

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Extracting Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Inhibitor: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C, protect form light.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Diluted GR solution: Add 6 µL GR into 0.12 mL deionized water before use. Store at 4°C, protected from light.

Diluted GR Cofactor Solution: Add 1.25 mL of deionized water into each GR Cofactor before use. Store at -20°C, protected from light.

Diluted Chromogen Solution: Add 1.25 mL of deionized water into each Chromogen before use. Store at 4°C, protected from light.

Standard preparation:

Diluted Extraction Buffer: Make a 1:10 dilution of the Extraction Buffer solution with deionized water in a clean plastic tube by diluting 250 µL Extraction Buffer into 2,250 µL deionized water.

20 mM GSSG Standard: Take 1 vial Standard and dissolve with 1 mL Diluted Extraction Buffer.

20 µM GSSG Standard: Prepare 20 µM GSSG Standard by diluting 1 µL 20 mM GSSG Standard into 999 µL Diluted Extraction Buffer. Using 20 µM GSSG Standard prepare standard curve as described:

Num.	20 µM Standard (µL)	Diluted Extraction Buffer (µL)	Concentration (µM)
Std.1	100	0	20
Std.2	80	20	16
Std.3	60	40	12
Std.4	40	60	8
Std.5	20	80	4
Std.6	10	90	2
Std.7	5	95	1
Blank	0	100	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for 10 days.

1. Animal Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Plasma or Serum: Collect plasma or serum using an anticoagulant. Centrifuge at 600 g for 10 min at 4°C. Collect

supernatant within 30 min and add equal volume of Extraction Buffer. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

4. Blood Cells: Collect blood using an anticoagulant. Centrifuge at 600 g for 10 min at 4°C. Discard the upper plasma, then wash the pellet with triple volume of cold PBS 3 times (use PBS resuspend blood cells, centrifuge at 600 g for 10 min at 4°C). Add equal volume of Extraction Buffer, then mix and stand at 4°C for 10 min. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

5. Cells or Bacteria: Collect 2×10^6 cells or bacteria for each assay. Wash cells or bacteria with cold PBS twice (resuspend with PBS, centrifuge at 600 g for 10 min at 4°C). Resuspend in triple volume of cells or bacteria pellet Extraction Buffer, repeated freeze-thaw cycles 2-3 times (can be frozen in liquid nitrogen, dissolved in 37°C water bath). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	2
Deionized Water	20	0	18
Different Concentrations Standard	0	20	0
Inhibitor	1	1	1
Mix well, incubate for 30 min at 37°C.			
Assay Buffer	140	140	140
Diluted GR Solution	2	2	2
Diluted GR Cofactor Solution	20	20	20
Diluted Chromogen Solution	20	20	20

3. Mix well, immediately detect optical density at 412 nm as A_1. Incubate for 10 min at 37°C in the dark. Measure optical density of 10 min at 412 nm again as A_2, $\Delta A = A_2 - A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} value of Samples are higher than the $\Delta A_{Standard}$ value of the 200 μM Standard, dilute sample with deionized water and repeat this assay.

Data Analysis

The measured absorbance values of standard well and test well should minus the absorbance of blank well, that is, $\Delta\Delta A_{Standard} = \Delta A_{Standard} - \Delta A_{Blank}$, $\Delta\Delta A_{Test} = \Delta A_{Test} - \Delta A_{Blank}$.

1. Drawing the standard curve:

With the concentration of the Standard Solution as the y-axis and the $\Delta\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the $\Delta\Delta A_{Test}$ into the equation to obtain the y value (μM).

2.Calculate the content of GSSG in sample

(1) By sample fresh weight

GSSG (nmol/g)=y×V_{sample}÷(V_{sample}÷$V_{Extraction}$ ×W)×n=**y÷W×n**

(2) Calculated by protein concentration

GSSG (nmol/mg prot)=y×V_{sample}÷(V_{sample}×Cpr)×n=**y÷Cpr×n**

(3) Calculated by cells or bacteria number

GSSG (nmol/10^4)=y×V_{sample}÷(V_{sample}÷$V_{Extraction}$ ×cells or bacteria number)×n=y÷500×n=**0.002×y×n**

(4) Calculated by liquid volume

GSSG (nmol/mL)=**y×2×n**

Where: W: sample weight, g; V_{sample}: Sample volume added, 2 μL; $V_{Extraction}$: Extraction Buffer volume added, 1 mL; n: Dilution factor, 10; Cpr: Supernatant protein concentration, mg/mL; 500: Total number of cells or bacteria, 5×10^6; 2: Double the dilution during liquid extraction.

Typical Data

Typical standard curve

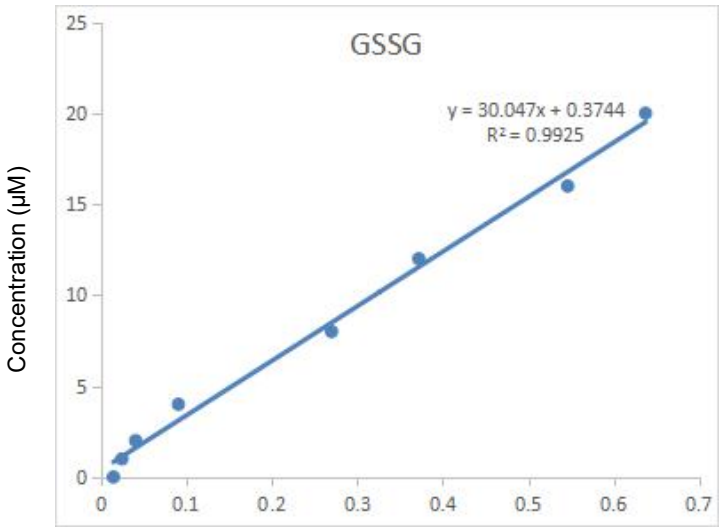

Figure 1. Standard Curve for GSSG.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.