EZMeta™ Glutathione S-Transferase (GST) Colorimetric Assay Kit

Cat #: D-AKC1630

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Detection range: 2-76 U/L

Sensitivity: 2 U/L

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria

Assay Principle

Glutathione S-transferases (GST) are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the products more water-soluble. The glutathione conjugates are metabolized further to mercapturic acid and then excreted. GST has the activity of GSH-Px, also called non-Se GSH-Px, which has the function of repairing oxidative damage of macromolecule such as DNA, protein and so on. GST catalyzed reaction decreased GSH content, but did not increase GSSG content. Based on their sequence homology, substrate specificity and immunological cross-reactivity, GSTs have been grouped into species-independent classes of isozymes. These classes are comprised of both cytosolic and microsomal enzymes. EZMeta™ Glutathione S-Transferase (GST) Colorimetric Assay Kit provides a simple method for detecting activity of GST in a variety of biological samples such as serum, plasma, animal tissues, cells and bacteria. GST catalyzes the binding of GSH to CDNB. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	50 mL	100 mL	4°C
Chromogen	10 mL	20 mL	4°C, protect form light
Substrate	1	1	4°C, protect form light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

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

·Ice maker, refrigerated centrifuge, incubator

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

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

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Substrate Working Reagent: Add 2 mL deionized water to dissolve Substrate before use. Store at 4°C, protected from light.

Sample Preparation

1. Animal or plant tissues: Weigh 0.1 g tissues, add 1 mL cold Assay 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. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Assay Buffer to ultrasonically disrupt the cells or bacteria 5 min on ice

(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. Serum and other liquids: Tested directly by adding samples to the microplate.

Note: The sample preparation should be carried out on ice. If the experiment is not carried out immediately, the samples can be kept at -80°C for 1 month. In the detection of GST activity in cells, the cell number must be between 3-5×10^6, and the extraction of GST in cells can be followed by Assay Buffer ultrasonic treatment, Cells cannot be treated by lysate. It will be better to quantify the total protein with EZMeta™ Glutathione S-Transferase (GST) Colorimetric Assay Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Substrate Working Reagent place at 25°C (for general species) or 37°C (for mammals) for 15 min.

3. Add the following reagents to the 96-well UV plate or microquartz cuvette:

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	20
Assay Buffer	20	0
Chromogen	180	180
Substrate Working Reagent	20	20

4. Mix Well. The absorbance values at 340 nm were measured. The blank well 10 s is recorded as A_1, 310 s is recorded as A_2, and the test well 10 s is recorded as A_3, 310 s is recorded as A_4, calculate $\Delta A_{Blank}=A_2-A_1$, $\Delta A_{Test}=A_4A_3$.

Note: Blank Well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the sample absorbance value is greater than 1, it is suggested that the sample should be diluted with Deionized Water and the result should be multiplied by the dilution factor. The reaction temperature has

influence on the result. Keep the temperature at 25°C (for general species) or 37°C (for mammals).

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A.96-well UV plates calculation formula as below

1. By protein concentration

Active unit definition: at 25°C or 37°C, 1 μmol/L CDNB per mL of protein per min was catalyzed to bind GSH.

GST (U/mg prot)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times 10^6\times V_{Total}\div(Cpr\times V_{Sample})\div T$=**$0.46\times(\Delta A_{Test}-\Delta A_{Blank})\div Cpr$**

2. By sample fresh weight

Active unit definition: at 25°C or 37°C,1 μmol/L CDNB per g of sample per min was catalyzed to bind GSH.

GST (U/g fresh weight)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times 10^6\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$=**$0.46\times(\Delta A_{Test}-\Delta A_{Blank})\div W$**

3. By cells or bacteria numbers

Active unit definition: at 25°C or 37°C,1 μmol/L CDNB per 10^4 cells or bacteria of sample per min was catalyzed to bind GSH.

GST ($U/10^4$)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times 10^6\times V_{Total}\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$=**$0.46\times(\Delta A_{Test}-\Delta A_{Blank})\div 500$**

4. By liquid volume

Active unit definition: at 25°C or 37°C, 1 μmol/L CDNB per mL of liquid per min was catalyzed to bind GSH.

GST (U/mL)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times 10^6\times V_{Total}\div V_{Sample}\div T$=**$0.46\times(\Delta A_{Test}-\Delta A_{Blank})$**

Where: ε: Molar extinction coefficient of the product, 9.6×10^3 L/mol/cm; d: Light diameter of a 96-well UV plate, 0.5 cm; 10^6: 1 mol=1×10^6 μmol; V_{Total}: Total volume of the reaction system, 220 μL=2.2×10^{-4} L; Cpr: Supernatant protein concentration, mg/mL; W: Sample quality, g; V_{Sample}: The volume of supernatant added to the reaction , 20 μL=0.02 mL; $V_{Sample\ Total}$: Volume of extraction solution, 1 mL; T: Reaction time, 5 min; 500: Number of cells or bacteria, 5×10^6.

B.Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Disclaimer

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