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Ver.250201

Glutathione S-transferase (GST) Activity Assay Kit

BC3309-02 (100 Tests/96 Samples)

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

Product Description

Glutathione S-transferase (GST) is a family of proteins with many physiological functions, which mainly exists in the cytoplasm. GST is an important part of detoxification enzyme system in the body. It mainly catalyzes various chemical substances and their metabolites to covalent bond with the sulfhydryl group of GSH. So that electrophilic compounds become hydrophilic substances, which are easy to be excreted from bile or urine, so as to degrade various potentially toxic substances in the body and expel them out of the body. Therefore, GST plays an important biological role in protecting cells from electrophilic compounds. In addition, because GST has the activity of GSH-Px, it is also called non-se GSH-px and has the function of repairing macromolecular such as DNA and protein damaged by oxidation. Note that GST-catalyzed reactions reduce GSH content but do not increase GSSG content.

GST catalyzed the binding of GSH with CDNB, and the light absorption peak wavelength of the binding product is 340 nm. Calculate the GST activity by measuring the absorbance rising rate at the wavelength of 340 nm.

Kit components

Reagent	Volume	Storage
Reagent I	110mL × 1	4°C
Reagent II	20mL × 1	4°C, in dark
Reagent III	Powder × 1	4°C, in dark
Reagent IV	9mL × 1	4°C

Reconstitution of Reagent III:

Transfer the entire contents of Reagent IV (9mL) to the bottle containing Reagent III (Powder). Dissolve completely, prepare aliquots of 500μL and store at -20°C.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, refrigerated centrifuge, water bath/constant temperature incubator, micro quartz cuvette/96 well UV flat-bottom plate, mortar/homogenizer, ice and distilled water.

Protocol

I. Sample Preparation

Tissue: Add 1mL Reagent I to 0.1g tissue. Homogenate in ice and centrifuge at 8000 rpm at 4°C for 10 minutes. Supernatant is used for the assay (Determine protein concentration of the supernatant).

Cells: Add 1mL Reagent I to 5 million cells. Lyse by subjecting to ultrasonication (power: 300W, sonication: 3 seconds, interval: 7 seconds) while keeping the tube in ice. Centrifuge at 8000 rpm at 4°C for 10 minutes. Supernatant is used for the assay (Determine protein concentration of the supernatant).

Serum/Plasma: Use directly.

II. Assay procedure

- Preheat the spectrophotometer/microplate reader for 30 min, adjust wavelength to 340 nm and set zero with distilled water.
- Reagent II and reconstituted Reagent III should be pre-heated to 37°C (mammal) or 25°C (others) for 30 minutes.

- **Blank tube:** Take a micro quartz cuvette or 96 well UV flat-bottom plate, add 20μL of Reagent I, 180μL of Reagent II and 20μL of Reagent III. Mix thoroughly, detect the absorbance (A_1) at 340 nm at the time of 10 seconds. Then place cuvette/96 well UV flat-bottom plate with the reaction solution at 37°C (mammal) or 25°C (general species) for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A_2 .
- **Test Tube:** Take a micro quartz cuvette or 96 well UV flat-bottom plate, add 20μL of Supernatant, 180μL of Reagent II and 20μL of Reagent III. Mix thoroughly, measure the absorbance at 340 nm at the time of 10 seconds record as A_3 . Then place cuvette/96 well UV flat-bottom plate with the reaction solution at 37°C (mammal) or 25°C (general species) for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A_4 .

Calculations

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the combination of 1μmol of CDNB with GSH in the reaction system at 37°C or 25°C per minute for every milligram of protein.

$$\begin{aligned}\text{GR (U/mg protein)} &= [(A_4 - A_3) - (A_2 - A_1)] \div (\epsilon \times d) \times V_{RV} \times 10^6 \div [\text{Cpr} \times V_{RS}] \div T \\ &= 0.23 \times [(A_4 - A_3) - (A_2 - A_1)] \div \text{Cpr}\end{aligned}$$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the combination of 1μmol of CDNB with GSH in the reaction system at 37°C or 25°C per minute for every gram of tissue.

$$\begin{aligned}\text{GR (U/g weight)} &= [(A_4 - A_3) - (A_2 - A_1)] \div (\epsilon \times d) \times V_{RV} \times 10^6 \div [V_{RS} \div V_{S1} \times W] \div T \\ &= 0.23 \times [(A_4 - A_3) - (A_2 - A_1)] \div W\end{aligned}$$

3. Serum/Plasma:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the combination of 1μmol of CDNB with GSH in the reaction system at 37°C or 25°C per minute for every millilitre of serum/plasma.

$$\begin{aligned}\text{GR (U/mL)} &= [(A_4 - A_3) - (A_2 - A_1)] \div (\epsilon \times d) \times V_{RV} \times 10^6 \div V_{RS} \div T \\ &= 0.23 \times [(A_4 - A_3) - (A_2 - A_1)]\end{aligned}$$

4. Cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the combination of 1μmol of CDNB with GSH in the reaction system at 37°C or 25°C per minute for every 10⁴ cells.

$$\begin{aligned}\text{GR (U/10}^4\text{ cells)} &= [(A_4 - A_3) - (A_2 - A_2)] \div (\epsilon \times d) \times V_{RV} \times 10^6 \div V_{RS} \div T \\ &= 0.23 \times [(A_4 - A_3) - (A_2 - A_2)] \div 500\end{aligned}$$

Cpr	:	Sample protein concentration (mg/ml)
W	:	Sample weight in grams.
ε	:	Molar extinction coefficient for the product, 9.6×10 ³ L/mol/cm.
10 ⁶	:	Unit conversion coefficient; 10 ⁶ μmol
V _{RS}	:	Volume of sample; 0.1mL
V _{RV}	:	Total volume of the reaction system, 1100 μL=1.1×10 ⁻⁴ L
V _{S1}	:	Volume of reagent I; 1mL
T	:	Reaction time, 5 minutes

Notes:

- The sample processing and other processes shall be carried out on ice, and the enzyme activity should be measured on the same day. The homogenate shall not be frozen and thawed repeatedly.
- If the absorbance of the sample greater than 1, dilute the sample with distilled water, and calculate result multiplied by dilution ratio.