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Glutathione S-transferase (GST) Activity Assay Kit

BC4405-01 (50 Tests/48 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	60mL × 1	4°C
Reagent II	50mL × 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

Constant temperature water bath, spectrophotometer, cooling centrifuge, 1mL quartz cuvette 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 1mL quartz cuvette, add 100μL of Reagent I, 900μL of Reagent II and 100μL of reconstituted Reagent III. Mix thoroughly, detect the absorbance (**A₁**) at 340 nm at the time of 10 seconds. Then place cuvette 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 **A₂**.
- Test Tube: Take a 1 mL quartz cuvette, add 100μL of supernatant, 900μL of Reagent II and 100μL of reconstituted Reagent III. Mix thoroughly, measure the absorbance at 340 nm at the time of 10 seconds record as **A₃**. Then place cuvette 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 **A₄**.

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.

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

$$= 0.23 \times [(\Delta A_4 - \Delta A_3) - (\Delta A_2 - \Delta A_1)] \div \text{Cpr}$$

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.

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

$$= 0.23 \times [(\Delta A_4 - \Delta A_3) - (\Delta A_2 - \Delta A_1)] \div W$$

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.

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

$$= 0.23 \times [(\Delta A_4 - \Delta A_3) - (\Delta A_2 - \Delta A_1)]$$

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.

$$\text{GR (U/10⁴ cells)} = [(\Delta A_4 - \Delta A_3) - (\Delta A_2 - \Delta A_2)] \div (\epsilon \times d) \times V_{RV} \times 10^6 \div V_{RS} \div T$$

$$= 0.23 \times [(\Delta A_4 - \Delta A_3) - (\Delta A_2 - \Delta A_2)] \div 500$$

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

Note

- 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.