# Glutathione S-transferase (GST) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC0350

**Size:** 50T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

| Reagent name | Size | Preservation Condition |
|---|---|---|
| Reagent I | Liquid 60 mL×1 | 2-8℃ |
| Reagent II | Liquid 55 mL×1 | 2-8℃ |
| Reagent III | Powder×1 | 2-8℃ |

**Solution Preparation**:

1. Reagent III: Dissolve with 6 mL of distilled water before use. Store at 2-8℃ for 4 weeks.

**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.

**Reagents and Equipment Required but Not Provided.**

Spectrophotometer, refrigerated centrifuge, water bath/ constant temperature incubator, transferpettor, 1 mL quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

**Procedure:**

**I. Extraction of crude enzyme solution:**

1. Tissue:

According to the tissue weight (g): Reagent I volume (mL) is 1:5-10 (it is recommended that add 1 mL of Reagent I to 0.1 g of tissue) for ice bath homogenization. Centrifuge at 8000 ×g for 10

minutes at 4℃ to

remove insoluble materials and take the supernatant on ice for testing.

2. Bacteria or cells:

According to the number of bacteria or cells ($10^4$): Reagent I volume (mL) is the proportion of 500~ 1000: 1 (it is recommended that add 1 mL of Reagent I to 5 million bacteria or cells), and break the

bacteria or cells by ultrasound (placed on ice, ultrasonic power 300W, working time 3 seconds, interval 7 seconds, repeat for 18 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.

3. Serum (plasma):

Detect sample directly.

**II. Procedure**

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and adjust the zero with distilled water.
2. Keep the Reagent II warm at 37℃ while in use.
3. Blank tube: Take a 1 mL quartz cuvette, add 100 μL of Reagent I, 900 μL of Reagent II and 100 μL of Reagent III. Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A1. Then place cuvette with the reaction solution in a 37℃ water bath/constant temperature incubator for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2.
4. Test Tube: Take a 1 mL quartz cuvette, add 100 μL of supernatant, 900 μL of Reagent II and 100 μL of Reagent III. Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A3. Then place cuvette with the reaction solution in a 37℃ water bath/constant temperature incubator for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A4.

**III. Calculation of GST activity**

1. Calculate by sample protein concentration

Unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37℃ per minute every milligram protein.

GST(U/mg prot) $=[(A4-A3)-(A2-A1)]\div(\varepsilon\times d)\times10^6\times Vrv\div(Cpr\times Vrs)\div T$

$=0.23\times[(A4-A3)-(A2-A1)]\div Cpr$

2. Calculate by fresh sample weight

Unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37℃ per minute every gram tissue sample.

GST(U/g fresh weight) $=[(A4-A3)-(A2-A1)]\div(\varepsilon\times d)\times10^6\times Vrv\div(Vrs\div Vs_1\times W)\div T$

$=0.23\times[(A4-A3)-(A2-A1)]\div W$

3. Calculate by cell amount

Unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the

combination of 1 μmol of CDNB with GSH in the reaction system at 37℃ per minute every $10^4$ cells.

GST(U/104 cell) =[(A4-A3)-(A2-A1)]÷(ε×d)×$10^6$×Vrv ÷(500×Vrs÷$Vs_1$)÷T

=0.23×[(A4-A3)-(A2-A1)] ÷500

4. Calculate by liquid volume

Unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37℃ per minute every milliliter

liquid sample.

GST (U/mL)= [(A4-A3)-(A2-A1)]÷(ε×d)×$10^6$×Vrv ÷Vrs÷T

=0.23×[(A4-A3)-(A2-A1)]

ε：Molar extinction coefficient for the product, 9.6×$10^3$ L/mol/cm.

d: Light diameter of the cuvette, 1 cm;

$10^6$:1 mol=1×$10^6$ μmol;

Vrv: Total volume of the reaction system, 1100 μL=1.1×$10^{-4}$ L;

Cpr: Supernatant protein concentration (mg/mL).

Vrs: Add supernatant liquid volume into the reaction system, 100 μL =0.1 mL;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

$Vs_1$: Volume of Reagent I, 1 mL;

N: The number of cells, tens of thousands.

**Note:**

1. Sample preparation processes should be operated on the ice, and enzyme activity must be measured on the same day.
2. For cell sample test, keep cell amount between 3-5 million. The extraction of GST in cells can be followed by grinding or ultrasonic treatment with Reagent I, but not treated with cell lysate.
3. If the absorbance of the sample greater than 1, dilute the sample with distilled water, and calculate result multiplied by dilution ratio.
4. Reaction temperature could infect determination result, general specie samples operated at 37℃.

**Experimental Examples：**

1. Take 0.1 g of rose, add 1 mL of Reagent I, homogenize in ice bath, centrifuge at 4℃ and 8000g for 10min, take the supernatant, dilute 50 times, put it on ice for testing, operate according to the determination steps, and calculate $\Delta A_T$ = A4-A3 = 0.647-0.587 = 0.06, $\Delta A_B$= A2-A1 = 0.591-0.539 = 0.052
GST (U/g mass) = 0.23 × [(A4-A3) - (A2-A1)] ÷W × 50 (dilution ratio) = 0.92 U/g mass.

2. Take 0.1g of liver, add 1ml Reagent I, homogenize in ice bath, centrifuge at 4℃ and 8000g for 10 min, take the supernatant, dilute 500 times, and place it on ice for measurement. Operate according to the determination steps, and calculate $\Delta A_T$= A4-A3 = 0.824-0.543 = 0.281, $\Delta A_B$= A2-A1 = 0.591-0.539 = 0.052
GST (U/g mass) = 0.23 × [(A4-A3) - (A2-A1)] ÷ W × 500 (dilution ratio) = 263.35 U/g mass.

**Recent Product Citations:**

[1] Zeeshan M, Hu YX, Guo XH, Sun CY, Salam A, Ahmad S, Muhammad I, Nasar J, Jahan MS, Fahad S, Zhou XB. Physiological and transcriptomic study reveal SeNPs-mediated AsIII stress detoxification mechanisms involved modulation of antioxidants, metal transporters, and transcription factors in Glycine max L. (Merr.) roots. Environ Pollut. 2023 Jan 15; 317:120637. doi: 10.1016/j.envpol.2022.120637. Epub 2022 Nov 16. PMID: 36400144.

[2] Zhu H, Yan C, Yao P, Li P, Li Y, Yang H. Ginsenoside Rg1 protects cardiac mitochondrial function via targeting GSTP1 to block S-glutathionylation of optic atrophy 1. Free Radic Biol Med. 2023 Aug 1; 204:54-67. doi: 10.1016/j. freeradbiomed.2023.04.015. Epub 2023 Apr 25. PMID: 37105420.

[3] Cheng C, Zhang J, Liu K, Xu Y, Shen F, Han Y, Hou Y, Zhang T, Bai G. Ginsenoside CK targeting KEAP1-DGR/Kelch domain disrupts the binding between KEAP1 and NRF2-DLG motif to ameliorate oxidative stress damage. Phytomedicine. 2023 Oct; 119:154992. doi: 10.1016/j.phymed.2023.154992. Epub 2023 Jul 23. PMID: 37499433.

**References:**

[1] Santillán-Sidón P, Pérez-Morales R, Anguiano G. et al. Glutathione S-transferase activity and genetic polymorphisms associated with exposure to organochloride pesticides in Todos Santos, BCS, Mexico: a preliminary study [J]. Environmental Science and Pollution Research, 2020, 27(34): 43223-43232.

[2] Martins L, Teixeira J. Gene- and organ-specific impact of paracetamol on Solanun nigrum L.'s γ-glutamylcysteine synthetase and glutathione S-transferase and consequent phytoremediation fitness[J]. Acta Physiologiae Plantarum, 2021, 43: 53.

[3] Taysi M.Ş., Temel Y. Glutathione S-transferase: Purification and Characterization from Quail (Coturnix coturnix japonica) Liver and the Impact of Some Metal Ions on Enzyme Activity[J]. BioNanoScience, 2021, 11:91-98.

**Related Products:**

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|---|---|
| BC1170/BC1175 | Reduced Glutathione(GSH) Content Assay Kit |
| BC1180/BC1185 | Oxidized Glutathione(GSSG) Content Assay Kit |
| BC1190/BC1195 | Glutathione Peroxidase(GPX) Activity Assay Kit |
| BC1160/BC1165 | Glutathione Reductases(GR) Activity Assay Kit |
| BC1150/BC1155 | Oxidized Thioredoxin Reductase(TrxR) Activity Assay Kit |
| BC1210/BC1215 | γ-glutamylcysteine Ligase(GCL) Activity Assay Kit |
| BC1220/BC1225 | γ-glutamyl Transpeptidase(γ-GT) Activity Assay Kit |