Glutathione Reductase (GR)Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC1165

Size: 100T/96S

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 125 mL×1	2-8℃
Reagent II	Powder×1	2-8℃
Reagent III	Powder×1	2-8℃

Solution Preparation:

1. Reagent II: Before use, add 1.5mL distilled water to dissolve and set aside. Store at 2-8℃ for 4 weeks.

2. Reagent III: Reagents are stored in glass bottles inside reagent bottles; Before use, add 3mL distilled water to dissolve and set aside. It can be stored separately at -20℃ for 4 weeks to avoid repeated freezing and thawing.

Product Description:

GR is a flavor-protein oxidoreductase widely existing in eukaryotes and prokaryotes. GR catalyzes the reduction of GSSG to GSH, which is one of the key enzymes of glutathione redox cycle (GR is usually replaced by TrxR in insects). GR catalyzes the reduction of GSSG to generate GSH by NADPH, which is helpful to maintain the body's GSH/GSSG ratio.GR plays a key role in the scavenging of reactive oxygen species in oxidative stress. In addition, GR also participates in thecycle pathway of ascorbic acid and glutathione.

GR catalyzes the reduction of GSSG by NADPH to produce GSH, at the same time, NADPH dehydrogenation produces $NADP^+$. NADPH has a characteristic absorption at 340 nm. On the contrary, $NADP^+$ has no absorption peak at this wavelength. The rate of NADPH dehydrogenation is determined by measuring the rate of decrease of absorbance at 340 nm, thereby calculating GR activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, low temperature centrifuge, water bath, adjustablepipette, micro quartz cuvette/96 well flat-bottomplate (UV plate) and distilled water.

Procedure

I. Crude enzyme extraction:

1. **Tissue:** according to the tissue weight (g): the volume of the Extract solution (mL) is 1:5-10. It is

suggested that add 1 mL of Reagent I to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 rpm 4℃ for 10 minutes. Take the supernatant on ice for test.

2. **Cells:** according to the number of the cells (10^4): the volume of the Extract solution (mL) is 500~1000:1. It is suggested that add 1 mL of Reagent I to 5 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 10000 rpm 4℃ for 10 minutes. Take the supernatant on ice for test.
3. **Serum(plasma):** detect directly.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. The Reagent I is preheated in 37℃ for greater than 30 minutes.
3. Blank tube: Take micro quartz cuvette/96 well flat-bottom plate (UV plate), add 10 μL of Reagent II, 20 μL of Reagent III, 170 μL of Reagent I, measure the absorbance at 340 nm for 10s and 190s, record as A_{B1} and A_{B2}.
4. Test tube: Takemicro quartz cuvette/96 wellflat-bottom plate (UV plate), add 10 μL of Reagent II, 20 μL of Reagent III, 20 μL of supernatant, 150 μL of Reagent I, measure the absorbance at 340 nm for 10s and 190s, record as A_{T1} and A_{T2}.

Note: after measuring the absorbance of the sample for 10s, put the cuvette into a 37℃water bath, take out the cuvette after 3 minutes, mix it well, and immediately measure the absorbance at 190s.

III. Calculation:

A. The calculation formula for the determination of micro quartz cuvette.

1. Calculation of GR activity

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADPH per min at a certain temperature and pH 8.0 every milligram of protein.

GR (U/mg prot)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div[Cpr\times V_S]\div T=0.536\times(\Delta A_T-\Delta A_B)\div Cpr$

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADPH per min at a certain temperature and pH 8.0 every gram of sample.

GR (U/g weight)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_S\div V_{SV}\times W)\div T=0.536\times(\Delta A_T-\Delta A_B)\div W$

3) serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADPH per min at a certain temperature and pH 8.0 every mL serum.

GR (U/10^4 cell)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_S\div V_{SV}\times N)\div T=0.536\times(\Delta A_T-\Delta A_B)\div N$

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of NADPH per min at a certain temperature and pH 8.0 every 10^4 cell.

GR (U/g weight)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div V_S\div T=0.536\times(\Delta A_T-\Delta A_B)$

$\Delta A_B=\Delta A_{B1}-\Delta A_{B2;}$

$\Delta A_T=\Delta A_{T1}-\Delta A_{T2};$

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Cuvette optical diameter, 1 cm;

V_{RV}: Total volume of reaction system, 200 μL = 2×10^4 L;

10^6: Unit conversion coefficient, 1 mol = 10^6μmol;

Cpr: Supernatant protein concentration, mg/mL;

V_S: Volume of supernatant added into reaction system, 20 μL = 2×10^2 mL;

V_{SV}: Volume of extract solution, 1 mL;

T: Reaction time , 3 minutes;

W: Sample weight, g。

N: Numbers of cells or bacteria (unit: 10^4);

B. The calculation formula for the determination of 96 well plate (UV plate).

Change the d-1cm in the above formula to d-0.6cm (light path of 96 well UV plate) for calculation

Note:

1. The sample processing and other processes shall be carried out on ice, and the enzyme activity shall be measured on the same day. The homogenate shall not be frozen and thawed repeatedly.
2. 1-2 samples should be used for pretest before the determination, and mammalian tissues should be diluted 2-5 times with Reagent I.
3. Because the Extract solution contains a certain concentration of protein (about 1mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

Experimental instances:

1. Take 0.1g of Peach leaves, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant, dilute 4 times and place it on ice for test according to the measured steps. Calculate $\Delta A_T=A_{T1}-A_{T2}=1.0765-0.626=0.4505$， $\Delta A_B=A_{B1}-A_{B2}=0$, calculate the enzyme activity according to sample weight:
GR activity (U/g weight)=$0.536\times(\Delta A_T-\Delta A_B)\div W\times4$（dilution ratio）=9.66 U/g weight.
2. Take 0.1g of rat liver, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant, dilute 8 times and place it on ice for test according to the measured steps. Calculate $\Delta A_T=A_{T1}-A_{T2}=0.9916-0.5632=0.4284$，$\Delta A_B=A_{B1}-A_{B2}=0$, calculate the enzyme activity according to sample weight: GR activity (U/g weight)=$0.536\times(\Delta A_T-\Delta A_B)\div W\times8$（dilution ratio）=18.37 U/g weight.

Recent Product citations

[1] Hua Li,LanyingWang,Yanping Luo. Composition Analysis by UPLC-PDA-ESI (−)-HRMS and Antioxidant Activity Using Saccharomyces cerevisiae Model of Herbal Teas and Green Teas from Hainan. Molecules. October 2018;(IF3.06)

[2] ZeyongZhang,HuanhuanLiu,CeSun,et al. A C2H2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice. Journal of Plant Physiology.October 2018;(IF2.825)

[3] Li S, Tian Y, Wu K, et al. Modulating plant growth–metabolism coordination for sustainable agriculture[J]. Nature, 2018, 560(7720)：595-600.

Reference:

[1] Demiral T, Türkan I. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance[J]. Environmental and experimental botany, 2005, 53(3): 247-257.

Related products：

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BC1210/ BC1215	γ-glutamate-cysteine ligase (GCL) Assay Kit
BC1220/ BC1225	γ-glutamyl transpeptidase (γ-GT) Assay Kit
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