Glutathione Reductase (GR)Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1160

Size: 50T/48S

Components:

Reagent I: 100 mL×1,Storage at 4℃.

Reagent II: 3 mL×1,Storage at 4℃.

ReagentIII: Powder×1, Storage at 4℃. Add 5.0 mL of distilled water before use and mix well.

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 the cycle 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,low temperature centrifuge, water bath,adjustable pipette, 1 mL quartz cuvette and distilled water.

Procedure

I. Crude enzyme extraction:

Suggested 0.1 g of tissue with 1mL of ReagentI， homogenate on ice bath.Centrifuge at 10000 rpm for 10minutes at 4℃, take the supernatant for test.

II. Determination procedure:

1. Preheat Spectrophotometer for 30minutes,adjust wavelength to 340 nm,set zero with distilled water.
2. The reagent I is preheated in 25℃ (common substance) or 37℃ (mammal) for greater than 30 minutes.
3. Blank tube: Take 1 mL of quartz cuvette, add 50 μL of Reagent II, 100 μL of Reagent III, 850 μL of Reagent I, measure the absorbance at 340 nm for 10s and 190s, record as A_{B1} and A_{B2}.
4. Test tube: Take 1 mL of quartz cuvette, add 50μLReagent II, 100 μL Reagent III, 100 μL

supernatant,750 μL 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 25℃(common substance) or 37℃(mammal) water bath, take out the cuvette after 3 minutes, mix it well, and immediately measure the absorbance at 190s.

III. Calculation:

1. Calculation of GR activity

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as an 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 an 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)= $[(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RV}\times 10^6]\div(V_S\div V_{SV}\times W)\div T$

$=0.536\times(\Delta A_T-\Delta A_B)\div W$

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

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

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, 1000 μL = 0.001 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, 100 μL = 0.1 mL;

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

T: Reaction time, 3 minutes;

W: Sample weight, g。

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. Reagent III shall be prepared and used now. After preparation, it shall be placed on ice;
3. 1-2 samples should be used for pretest before the determination, and mammalian tissues should be diluted 2-5 times with Reagent I.
4. 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$\Delta AT=AT1-AT2=1.033-0.953=0.08$， $\Delta AB=AB1-AB2=0.768-0.762=0.006$, 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 ridio）=1.587 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$\Delta A_T=A_{T1}-A_{T2}=0.886-0.533=0.353$， $\Delta A_B=A_{B1}-A_{B2}=0.768-0.762=0.006$, 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 ridio）=14.88 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：

BC1150/ BC1155	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
BC1210/ BC1215	γ-glutamate-cysteine ligase (GCL) Assay Kit
BC1220/ BC1225	γ-glutamyl transpeptidase (γ-GT) Assay Kit
BC1170/ BC1175	Reduced Glutathione (GSH) Assay Kit