SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Glutathione Reductase (GR)Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0479

Size: 100T/96S

Components:

Reagent I: 120 mL×1,Storage at 4℃ .

Reagent II: 1 mL×1,Storage at 4℃ .

ReagentIII: Powder×1, Storage at 4℃ . Add 2.0 mL of distilled water before use and mix well.

Product Description:

GR is aflavor-proteinoxidoreductasewidely 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:

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/microplate readerfor 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 micro quartz cuvette/96 well flat-bottomplate (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 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:

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 theamountofenzymecatalyzes the oxidation of 1 μmol ofNADPH per min at a certain temperature and pH 8.0every 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 theamountof enzyme catalyzes the oxidation of 1 μmol ofNADPH per min at a certain temperature and pH 8.0every 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, 200 μL = 2×10^4 L;

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

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。

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

2. Calculation of GR activity

1) Protein concentration:

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

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

$=0.893\times(\Delta A_T-\Delta A_B)\div Cpr$

2) Sample weight

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

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

$=0.893\times(\Delta A_T-\Delta A_B)\div W$

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

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

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

d: 96 well plate optical diameter, 0.6 cm;

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

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

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。

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 $\Delta A_T=A_{T1}-A_{T2}=1.0765-0.626=0.4505$， $\Delta A_B=A_{B1}-A_{B2}=0$, 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：

AK0482/ AK0481	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
AK0472/ AK0471	γ-glutamate-cysteine ligase (GCL) Assay Kit
AK0470/ AK0469	γ-glutamyl transpeptidase (γ-GT) Assay Kit
AK0478/ AK0477	Reduced Glutathione (GSH) Assay Kit