Elabscience®

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Glutathion Reductases (GR) Activity Assay Kit

Catalog No: E-BC-K290
Method: Colorimetric method
Specification: 96T **(**Can detect 94 samples without duplication)
Measuring instrument: Microplate reader, Spectrophotometer

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used to measure GR activity in tissue, cells, red blood cell lysates and other samples.

Detection significance

Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. Glutathione reductase functions as dimeric disulfide oxidoreductase and utilizes an FAD prosthetic group and NADPH to reduce one molar equivalent of GSSG to two molar equivalents of GSH.

Detection principle

Oxidized glutathione (GSSG) can be reduced to GSH under the catalysis of GR. GSH react with substrate DTNB to produce yellow TNB and GSSG. The production of TNB can be measured by absorbance at 412 nm. GR activity can be calculated indirectly by measuring the OD value at 412 nm.

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH$$

$$2GSH + DTNB \longrightarrow GSSG + 2TNB$$

Kit components

	Components	Specifications	Storage
Reagent 1	GR Test Buffer	50 mL ×1 vial	-20℃, 12 months
Reagent 2	Sample Diluent	50 mL ×1 vial	-20℃, 12 months
Reagent 3	NADPH	5 mg ×1 vial	-20℃, 12 months
Preparation of 6 mmol/L NADPH solution: dissolve a vial of powder with 1 mL deionized water and mix fully. It is recommended to aliquot the prepared solution and store at -80℃.			
Reagent 4	Oxidized glutathione(GSSG)	14.2 mg ×1 vial	-20℃, 12 months
Preparation of Reagent 4 application solution: dissolve a vial of powder with 10 mL deionized water and mix fully. It is recommended to aliquot the prepared solution and store at -20℃.			
Reagent 5	DTNB	4.5 mg ×1 vial	-20℃, 12 months
Reagent 6	DMSO	1.5 mL ×1 vial	-20℃, 12 months
Preparation of Reagent 5 application solution: dissolve a vial of DTNB powder with 1.5 mL DMSO and mix fully. It is recommended to aliquot the prepared solution and store at -20℃.			

Storage: Store at -20℃ for 1 year. Please store the dissolved NADPH at -70℃, it can be keep at 4℃ for one day, the activity of DADPH will reduce above 10% after store at -20℃ for 1 week. After preparing GSSG and DTNB into solutions, the solutions should be divided and stored at -20 ℃.

Experimental instrument

Micropipettor, Vortex mixer, Spectrophotometer/Microplate reader (412 nm)

Sample preparation

1. **Tissue:** Mince the tissues to small pieces, then be weighed and homogenized in normal saline on ice, the volume of normal saline(mL): the weight of the tissue(g)=9:1. The tissue homogenate is centrifuged for 10 min at 2500~3000 g, collect the supernatant and carry out the assay immediately. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

2. **Cells:** Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10^6): PBS (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

3. **Red Blood Cell Lysates:**
   Collect at least 500 μL of whole blood using heparin as an anticoagulant, centrifuge the sample at 2500 g for 5 min. Discard the supernatant, wash sediment 3 times with cold normal saline. Add cold deionized water (5 times volume of cell sediment) and resuspend the sediment. Incubate at ice-bath for 10 min. Centrifuge at 4℃ with 8500 g for 10 min, take the supernatant for assay.
   Determine the concentration of supernatants with BCA or CBB (Coomassie brilliant blue). Recommend use 20-200 μg of extracted protein to take a pre-test. If activity of GR is too high, the supernatants should be diluted with sample diluents. If activity of GR is too low, it should take more proteins for the assay. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

Operation steps

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

1. Bring all reagents to room temperature for 30 min.
2. Add the reagents to a 96 wells microplate according to the following table, mix well.

	Blank well	Sample well
Reagent 4 application solution (μL)	100	100
Reagent 1 (μL)	90	70-90
Sample (μL)	0	0-20
6 mmol/L NADPH solution (μL)	10	10
Total volume (μL)	200	200

3. Add 6.6 μL of DTNB solution, mix fully.
4. Preheat the microplate or spectrophotometer to 25℃ and measure the OD value at 412 nm.

5. Record the OD value every 2 min, record at least 10 min.
   [Note] If the OD value of sample is relatively low, please prolong the incubation time, the increasing of absorbance and extension of time will have positive correlation in a certain range. It can measure the OD value every 10 min at this situation.
6. Calculate OD/min for each sample, then subtract OD/min of blank well.

Calculation of results

Definition: At 25 ℃ and pH 7.5, the enzyme amount that 1 μmol GSSG was deoxidized per minute is defined as 1 unit.

$$\text{GR Activity (mU/mg)} = \frac{\text{OD(sample)/min} - \text{OD(blank)/min}}{0.01415} \times \text{Dilution factor} \div \text{Concentration of protein(mg/mL)}$$

Note: the molar extinction coefficient of TNB is 14.5 L /mol/cm.

1 U=1000 mU

Note

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The valid of kit is 12 months.
4. Do not use components from different batches of kit.
5. The Detection principle of the kit involve redox reaction, all the oxidant and reluctant can influence the assay of the kit. Sodium sulfate, ammonium sulfate and ferricyanide will interfere with the determination of the kit. Please try to avoid.
6. Sample can be measured immediately after collection, also can be stored at -70℃ for later detection.
7. Please strict control the reaction temperature, or lead big error.
8. NADPH is not stable, please take the assay followed the manual, beware of lose the activity.
9. Please take safety precautions and follow the procedures of laboratory reagent operation. All waste liquid should be handled in accordance with the relevant rules of biosafety.