(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

γ-Glutamylcysteine Synthetase (γ-GCS) Activity Assay Kit

Catalog No.: BC00153

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	γ-Glutamylcysteine Synthetase (γ-GCS) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, cells
Assay Type	Quantitative
Detection Instrument	Visible spectrophotometer or Microplate reader (optimal detection wavelength 636 nm)

Product Introduction

Glutamic acid cysteine synthase (γ-GCS) is the rate-limiting enzyme in the synthesis of glutathione (GSH). Increased intracellular γ-GCS activity accelerates GSH synthesis, thereby increasing the concentration of GSH both inside and outside the cell. GSH is an important antioxidant in the body, playing a crucial role in maintaining the integrity of airway epithelial cells, protecting against lung damage and inflammation, and combating oxidative damage. The lungs have high concentrations of γ-GCS enzyme activity and GSH (6.1–17.5 nmol/mg lung tissue), and the concentration of GSH in the respiratory epithelial lining fluid (200–400 μmol/L) is 100 times that in blood plasma. Therefore, GSH plays a very important role in respiratory inflammation and allergic reactions, and has significant research value in the treatment and prognosis of cardiovascular diseases and tumors.

Principle

Glutamate and cysteine, under the catalysis of gamma-glutamylcysteine synthase (γ-GCS), consume ATP and react to produce γ-glutamylcysteine, inorganic phosphorus, and ADP. The activity of gamma-glutamylcysteine synthase (γ-GCS) can be determined by measuring the amount of inorganic phosphorus in the system at 636 nm through a colorimetric reaction.

Components

Components	Name	Size	Storage
Reagent 1	Buffer solution	25 mL × 1 bottle	2~8℃
Reagent 2	Matrix solution	25 mL × 1 bottle	2~8℃
Reagent 3	Accelerator	Powder × 3 tubes	2~8℃
		Solvent 10 mL × 1 bottle	2~8℃
Preparation of reagent 3 application solution: Dissolve each vial of reagent 3 powder in 1.8 mL of reagent 3 solvent before use. Prepare and use immediately.			
Reagent 4	Terminator	6 mL × 1 bottle	RT
Reagent 5	Color developer	Solution A, 7 mL × 8 bottles	2~8℃
		Solution B, 6 mL × 8 bottles	2~8℃
Preparation of reagent 5 colorimetric reagent: Prepare reagent 5 A solution: reagent 5 B solution in a ratio of 7:6, store at 4℃ to prevent phosphorus contamination. (Note: Reagent 5 B solution may form flocculent precipitate at low temperatures. In this case, it can be dissolved by heating at 60℃ for about 10 minutes before use.)			
Reagent 6	Stabilizer	100 mL × 1 bottle	RT
Reagent 7	10 mmol/L standard phosphorus stock solution	5 mL × 1 bottle	2~8℃, away from light
Matrix buffer solution preparation: Prepare according to the ratio of Reagent 1: Reagent 2 = 0.25: 0.25. Prepare only the amount needed and prepare immediately before use. The mixed reagents are stable within 1 to 2 hours.			
Preparation of the colorimetric reagent: When using, add one bottle of reagent A to one bottle of reagent B, mix thoroughly, and store at 4°C. The prepared colorimetric reagent is enough for 13 tubes (if your sample volume is small, you can prepare the colorimetric reagent yourself according to the ratio of reagent A: reagent B = 7:6, prepare only the amount needed). The prepared colorimetric reagent can be stored at 4°C for at least five days.			
Preparation of 0.1 μmol/mL standard phosphorus working solution: When using, dilute the 10 mmol/L standard phosphorus stock solution 100 times with double-distilled water, that is, take 0.1 mL of the 10 mmol/L stock solution and add double-distilled water to 10 mL.			
Preparation of 0.02 μmol/mL phosphorus standard solution: When using, dilute 0.1 μmol/mL phosphorus standard solution with double-distilled water 5 times, that is, take 1 mL of 0.1 μmol/mL phosphorus standard solution and add 4 mL of double-distilled water.			

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Tissue: Accurately weigh the tissue and add physiological saline at a ratio of weight (g): volume (mL) = 1:9. Homogenize mechanically under ice-water bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant (i.e., the 10% homogenate supernatant). Dilute this supernatant 10 times with physiological saline to 1%. Simultaneously, determine tissue protein levels using Coomassie Brilliant Blue reagent (available at this store). If the preliminary test results are too high, dilute the 1% tissue homogenate to different concentrations and perform further preliminary tests to determine the optimal sampling concentration. Preparation of pancreatic supernatant: Accurately weigh pancreatic tissue, add 9 times the volume of homogenizing medium at a weight (g): volume (mL) ratio of 1:9, mechanically homogenize under ice bath conditions, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for analysis.
2. Cells: Digest the cultured cells, centrifuge, discard the supernatant, leaving a layer of cells. Add 0.2–0.3 mL of physiological saline or homogenizing medium to each tube to prepare a cell suspension of 10^7 / cm^3, i.e., 10^7 / mL, and then disrupt the cells. There are three methods for disrupting cells: ① Homogenize using a homogenizer. ② Disrupt using an ultrasonic homogenizer. ③ Thaw and repeatedly freeze-thaw three times (method ③ may sometimes affect enzyme activity). The prepared cell suspension does not need to be centrifuged. Simultaneously, measure tissue protein using Coomassie Brilliant Blue reagent (available in this reagent shop). Then dilute the cell homogenate to different concentrations for preliminary testing, and determine the sampling concentration based on the preliminary test results.

Note: 1. Shake well before adding the sample for testing.

2. Phosphate buffer or phosphorus-containing reagents should not be used as sample

homogenizers or for diluting samples.

3. The absolute absorbance value (absorbance value of the test tube - absorbance value of the control tube) should be controlled at around 0.2 in the preliminary test results.

Operation process

Reagents(μL)	Blank tube	Standard tube	Test tube	Control tube
Matrix buffer	--	--	250	250
Accelerator	--	--	25	25
Sample	--	--	25	--
37 ℃ water bath for 6 minutes.				
Terminator	--	--	25	25
Sample	--	--	--	25
Double-distilled water	325	--	--	--
0.02 μmol/mL phosphorus standard solution	--	325	--	--
Colorimetric reagent	1000	1000	1000	1000
Mix thoroughly and let stand at room temperature for 2 minutes.				
Stabilizer	1000	1000	1000	1000
After thorough mixing and standing at 37°C for 10 minutes, the sample was zeroed and colorimetrically analyzed using double-distilled water at a wavelength of 636 nm and a light path of 1 cm.				

Result calculation

The unit of ATPase activity is defined as the amount of inorganic phosphorus produced by ATPase from the breakdown of ATP by 1 μmol per milligram of tissue protein per hour. That is, μmol Pi/mgprot/hour.

$$\gamma-\text{GCS Activity}\ (\text{U/mgprot}) = \frac{A_{test}-A_{control}}{A_{standard}-A_{blank}} \times C_{standard} \times \frac{60}{T} \times 13 \div Cpr$$

Annotation

$C_{standard}$: Phosphorus standard solution concentration, 0.02 μmol/mL;

T: Reaction time, 6 min;

Cpr: Sample protein concentration, mgprot/mL (prot refers to protein).

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.