Glycerol Assay Kit

(Cat/No.:BC076 Size:100 T)

1. Product application

Measuring the glycerol concentration in solid tissues and cells.

2. Composition and preparation (3 months shelf life)

Reagent composition	Specifications	Save conditions
Lysis solution	100 ml × 1 bottle	This kit should be stored at 4℃ in the dark and has a validity period of 3 months.
R1	16 ml × 1 bottle	
R2	10 ml × 1 bottle	
4 mM glycerol standard solution	4 ml × 1 bottle	
Preparation of working solution: Mix 4 ml of reagent R1 with 1 ml of reagent R2 in the ratio of 4:1. Prepare it as needed and use it up immediately.		
[Note]: Be cautious of possible glycerol contamination from sources that are unknown but are prone to occur. Such contamination can come from the operator themselves or from the particle splashing of the standard solution. Discard the solution if it changes color.		
It can be used for 100 microplate determinations or 30 1 ml colorimetric cup determinations.		

3. Required equipment

Enzyme analyzer, (721, 722 models) visible light spectrophotometer, biochemical analyzer.
The optimal working wavelength is 550nm. If this wavelength is not available, it is recommended to choose 570nm, 530nm, or 490nm instead. The path length of the colorimeter cell is 1 cm.

4. Operation process

1.Sample processing

- **Organization of cell lysis:**

①.Cell lysis (including the breakdown of differentiated fat cells):

Digest and centrifuge the cells for collection. Or directly lysate them in the culture dish. Usually, in a 6-well plate, each well contains approximately $2x10^6$ cells, and in a $75mm^2$ flask, there are about $1x10^7$ cells. Add 0.1ml of lysis buffer to each $1\text{-}2x10^6$ cells in proportion and mix to perform lysis.

②、Animal tissue lysis:

Remember to weigh the fresh tissue beforehand and store it. Measuring the weight of the tissue after freezing and then thawing will result in significant measurement errors. Precisely weigh the centrifuge tube, add the fresh tissue, and then weigh again. Subtract the two values (i.e., the subtractive weighing method) to calculate the tissue weight (approximately 50mg). Add

10 μl of lysis buffer for every 1 mg of tissue. Using 0.5 - 1 ml of lysis buffer ensures effective lysis. Use an electric high-speed homogenizer or a manual glass homogenizer to break the tissue. The ultrasonic method is not recommended as it cannot completely and uniformly break the tissue. The amount of tissue cells added should be adjusted according to the pre-experiment.

- **Treatment with hydrolysis solution:**

  ①.Transfer 100 to 500 μl of the lysis buffer to a 1.5 ml centrifuge tube and proceed with the next step. The remaining lysis buffer can be used for protein quantification with the BCA protein quantification kit (A045-3) or stored at -20°C.

  ②.The lipase should be inactivated at 70℃ for 10 minutes.Flocculent precipitates may occur.

  ③.Centrifuge at room temperature at 5000 rpm for 5 minutes. The upper clear liquid can be used for enzymatic assays.

2.Standard dilution:

Using distilled water, normal saline or a liquid consistent with the sample buffer, dilute the 4 mM glycerol standard to 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 μmol/L in equal portions. Usually, 4 to 6 tubes are sufficient. Pay attention to setting up a control reaction tube with a 0 concentration.

3.Glycerol determination:

①.Refer to the table below for sample addition. The volume of the sample to be tested is 10 μl. Adding more samples can inhibit the reaction.

②.At 37°C or 25°C for 10 minutes. After the reaction reaches equilibrium, the color will remain stable within 60 minutes.

③.First, calibrate the instrument using a distilled water blank tube, and then measure the OD values of each tube.

④.Draw the standard curve and calculate the glycerol concentration. Steps for creating the graph in Excel: The OD values of each standard tube are on the y-axis, and the concentration of the standard sample is on the x-axis. ① Left-click on the data to select it, then click on the Chart Wizard, choose - Scatter Chart - and click - Finish -. ② Right-click on a certain point on the graph, then click on - Add Trendline -, click - Options -, click - Show Formula - and - R2 value -.

⑤.Correct the triglyceride content by using the concentration of protein per mg or the number of cells.

Add sample table

	96-well microplates			1ml colorimetric cup determination		
	Blank tube	Standard tube	Sample tube	Blank tube	Standard tube	Sample tube
Distilled water	10μl			35μl		
Standard sample		10μl			35μl	
Sample			10μl			35μl
Working solution	190μl	190μl	190μl	665μl	665μl	665μl

5. Product Description

Glycerol is the hydrolysis product of triglycerides. Just like free fatty acids, the content of glycerol is a reliable indicator for the hydrolysis reaction of triglycerides, but it is easier to detect. The kit adopts optimized steps to detect the content of glycerol in solid tissues and cells.

6. Determination principle

In the presence of ATP, glycerol is phosphorylated by glycerol kinase to 3-phosphoglycerol, which is then oxidized by glycerol phosphate oxidase to produce hydrogen peroxide. Under the action of peroxidase, the chromogenic substrate is converted to phenoxylidine, and the optical density value is proportional to the glycerol concentration.

7. Notes

1. If the reagent becomes cloudy or the OD value at 550nm of the blank tube is greater than 0.2, it should be discarded.

2. Vitamin C > 0.18g/L, Hemoglobin > 2g/L, Bilirubin > 0.25g/L, Dithiothreitol, Methylthioethanol, High Concentration EDTA interference test.

3. The measurement is affected by the synthesis of phosphoglycerol during red blood cell glycolysis.

8. References

1.Trinder, P. (1969). Ann. Clin. Biochem. 6:24 – 27.

2.Barham D and Trinder P. (1972). Analyst 97:142 – 145.