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Glycerol Assay Kit

Product Number: ARG-100T

Shipping and Storage

Stored at -20ºC, valid for one year. The Amplex Red and enzyme mixture must be stored away from light.

Component

Component	ARG-100T
Glycerol detection buffer	15mL
Amplex Red	200μL
glycerol kinase	200μL
Enzyme mixture	200μL
Cofactor	200μL
Glycerol standard solution (10mM)	200μL

Description

The Amplex Red Glycerol Assay Kit developed by our company is a probe based kit that uses fluorescence or absorbance detection to quickly and sensitively detect the glycerol content in serum, plasma, tissue or cell samples, urine, biological fluids, tissue or cell culture supernatants, and other samples. This kit only detects the content of free glycerol and does not detect the glycerol content in triglycerides.

Glycerol, also known as glycerol or glycerol, is a colorless, odorless, sweet, clear, viscous liquid. The molecular formula of glycerol is $C_3H_8O_3$, with a molecular weight of 92.09. The chemical structure of glycerol is different from that of carbohydrates and does not belong to the same category of substances. Each gram of glycerol can generate 4 calories when completely oxidized, and glycerol is usually absorbed by the human body without changing blood sugar and insulin levels. Glycerol is a commonly used sweetener and moisturizer in the food processing industry, mostly found in sports food and dairy substitutes. Due to its ability to increase the moisture content in human tissues, glycerol can enhance the body's ability to move in high heat environments. Glycerol is widely used in the production of food, beverages, solvents, drugs, and cosmetics, therefore its quantification is of great significance in research and development.

Glycerol is the main component of triglycerides, the most important storage method for fats, and an important metabolite produced during the oxidation and synthesis processes of energy metabolism. When the human body ingests fat, most of it is emulsified into small particles by bile. Lipases secreted by the pancreas and small intestine hydrolyze the fatty acids in the fat into free fatty acids and monoglycerides. After absorption, monoglycerides and long-chain fatty acids are re synthesized into triglycerides in small intestinal cells. Under physiological conditions, triglycerides are hydrolyzed by lipolysis to produce glycerol and free fatty acids (FFA), which are then released into the bloodstream. The generated glycerol cannot be reabsorbed and utilized by adipose tissue like fatty acids, so the levels of glycerol and free fatty acids in the blood are important indicators for measuring lipolysis levels and are also important indicators in the development of many related drugs.

The Amplex Red in this kit is a highly sensitive fluorescent probe for H2O2. In the presence of horseradish peroxidase (HRP), Amplex Red can react 1:1 with H2O2 to produce a strong red fluorescent substance called Resorufin. The maximum excitation wavelength of the test brine is 571nm, the maximum emission wavelength is 585nm, and there is strong visible light absorption at the excitation wavelength. Therefore, this kit can be tested using both fluorescence and absorbance methods.

The detection principle of this reagent kit is shown in Figure 1. In the presence of ATP, glycerol is phosphorylated by glycerol kinase (GK) to glycerol-3-phosphate. The generated glycerol-3-phosphate then undergoes an oxidation reaction with oxygen gas under the action of glycerol phosphate oxidase (GPO) to produce dihydroxyacetone phosphate (DHAP) and H_2O_2. The content of glycerol is finally detected by detecting the fluorescence intensity or absorbance of the reaction product of H_2O_2 and Amplex Red, chlorpromazine. The fluorescence intensity and absorbance of test brine are directly proportional to the content of glycerol in the

sample.

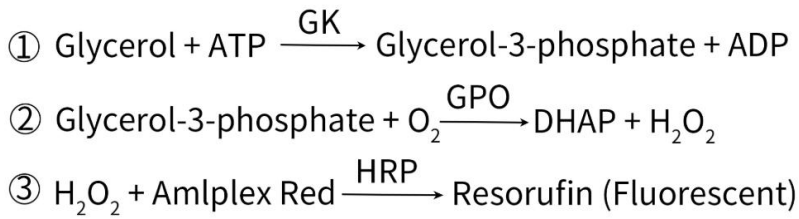

Figure 1. Schematic diagram of glycerol assay kit (ARG-100T) for detecting glycerol

This kit provides a standard solution of glycerol, which can be used to calculate the glycerol content in the sample by setting a standard curve. The detection effect of this reagent kit on glycerol standard is shown in Figure 2.

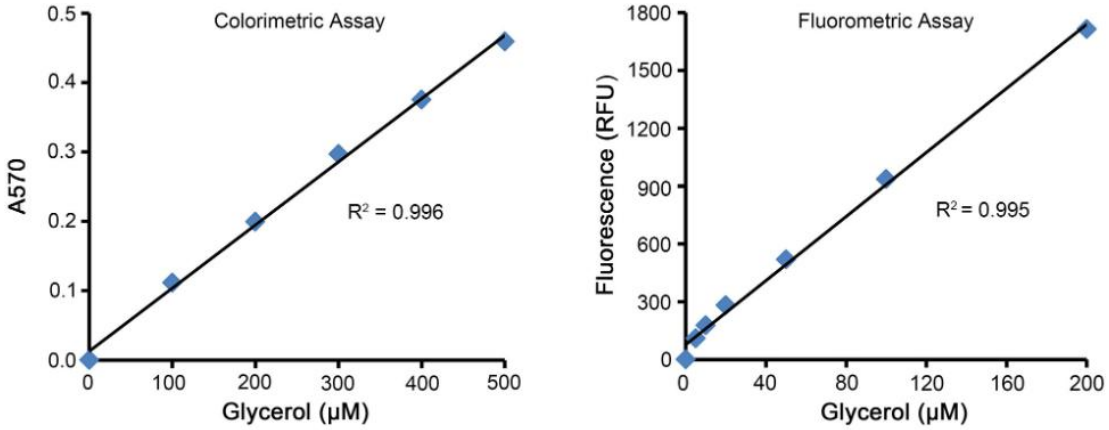

Figure 2. The standard curve for glycerol detection using the Glycerol Assay Kit (ARG-100T). The left image shows absorbance detection, and the right image shows fluorescence detection. The detection data in the figure shows a 60 minute reaction at 37ºC in the dark, and the value may be slightly lower after 30 minutes of reaction. The measured data may vary due to differences in experimental conditions, testing instruments, etc. The data in the figure is for reference only.

Protocol

1. Preparation of samples

1.1. Preparation of blood samples: For serum samples, place whole blood at room temperature, such as 25ºC, for 30 minutes to 2 hours without vigorous shaking to prevent hemolysis. After the whole blood naturally coagulates and the serum separates, centrifuge at 4ºC for about 1000-2000 × g for 10 minutes, and take the yellow supernatant to obtain the serum. Be careful not to extract white or light yellow precipitates; For plasma samples, anticoagulate whole blood with heparin or EDTA, centrifuge at 4ºC for about 1000-2000 × g for 10 minutes, and take the yellow or pale yellow supernatant to obtain plasma. Be careful not to extract the white precipitate. Serum and plasma should be placed on ice. If immediate detection is not possible, they can also be divided and stored at -20ºC or -80ºC for a short period of time. For frozen samples, they should be thawed before testing and stored in an ice bath for later use. They must be mixed thoroughly before use.

1.2. Preparation of cell or tissue samples: For cultured adherent cells, wash once with PBS and remove any remaining liquid. For cultured suspension cells, centrifuge appropriately (e.g. 100-500 × g, 5 minutes) to collect the cells into a centrifuge tube, discard the supernatant, and aspirate the residual liquid. Add 100-200μL of isopropanol at a ratio of every 200000 to 1 million cells and blow appropriately. For adherent cells, blow them appropriately to detach them from the culture vessel and transfer them to a centrifuge tube. For tissue samples, add 100-200μL of isopropanol per 10-20mg of tissue. For cultured cells and tissues, it is recommended to control the volume to 100-200μL using TissueMaster ™ High throughput tissue grinder (1.5/2mL × 48) , TissueMaster™ The handheld tissue grinder or glass homogenizer is used for homogenization under low temperature conditions such as about 4ºC or ice bath; Alternatively, the volume can be enlarged to around 400-500μL and homogenized using ceramic bead mechanical oscillation; Alternatively, conventional glass homogenizers can be used for homogenization (it is recommended to use smaller glass homogenizers as much as

possible to control the volume of the sample within a smaller range). Centrifuge at approximately 12000 × g at 4ºC for 3-5 minutes, and collect the supernatant for subsequent testing.

Note: The sample prepared with isopropanol should be diluted at least 5 times with detection buffer (i.e. the isopropanol content in 50μL of the test sample should not exceed 20%) before being used for subsequent detection; All the above operations must be performed at 4ºC or on ice. If the prepared cell or tissue samples cannot be detected immediately, they can be frozen at -20ºC or -80ºC; Isopropanol is prone to volatilization due to prolonged operation time. Subsequently, isopropanol can be replenished to the initial volume before sample detection; When the volatility of all samples is similar, it is not necessary to supplement the evaporated isopropanol uniformly, but the volume of evaporated isopropanol needs to be considered when calculating the concentration in the samples.

1.3. Preparation of cell culture supernatant samples: For adherent cells, directly aspirate the culture medium; For suspended cells, centrifuge and aspirate the culture medium.

2. Preparation of reagent kit

2.1. Dissolve glycerol standard solution (10mM) and detection buffer, equilibrate to room temperature, and mix well for later use. Other reagents should be stored in an ice bath for later use, and should be stored immediately according to the conditions required by the reagent kit after use.

2.2. Preparation of glycerol detection working solution: Prepare an appropriate amount of glycerol detection working solution according to a volume of 80μL for each detection reaction. Uniform mixing 72μL Glycerol Assay Buffer、2μL Amplex Red、2μL Glycerol Kinase、2μL Enzyme Mix、2μL Cofactor, It can be prepared into 80μL glycerol detection working solution. Prepare an appropriate amount of glycerol detection working solution based on the quantity of samples to be tested (including standard samples). Please refer to the table below for specific preparation methods. If the prepared testing solution is stored at 4ºC or in an ice bath away from light, it can be used on the same day, but it is recommended to prepare and use it as soon as possible.

Samples	1	10	20	50
Glycerol Assay Buffer (μL)	72	720	1440	3600
Amplex Red (μL)	2	20	40	100
Glycerol Kinase (μL)	2	20	40	100
Enzyme Mix (μL)	2	20	40	100
Cofactor (μL)	2	20	40	100
Working Solution (μL)	80	800	1600	4000

Note: Due to the small amount and easy settling of enzyme solution, it is necessary to gently centrifuge it before use, and then mix it properly before use.

3. sample determination

3.1. Glycerol standard curve setting (either absorbance detection or fluorescence detection, one can be selected. For situations with small sample sizes, fluorescence detection is preferred)

3.1.1. Absorbance detection: Take 10μL of glycerol standard solution (10mM), add 190μL of detection buffer (if detecting cell or tissue samples prepared with isopropanol, the same detection buffer as the isopropanol content in the sample can also be used more accurately, but the isopropanol content in 20μL of the standard should not exceed 50%), mix well, and prepare a glycerol standard solution with a concentration of 500μM. Take 0, 4, 8, 12, 16, and 20μL of 500μM glycerol standard solution and add them to the standard wells of a 96 well plate. Fill up to 20μL with detection buffer or a detection buffer containing an appropriate amount of isopropanol. At this point, the concentrations of the standard curves are 0, 100, 200, 300, 400, and 500 μM, respectively.

Note: It is recommended to use a transparent 96 well plate for absorbance detection.

3.1.2. Fluorescence detection: Take 4μL of glycerol standard solution (10mM), add 196μL of detection buffer (if detecting cell or tissue samples prepared with isopropanol, the same detection buffer as the isopropanol content in the sample can also be used more accurately, but the isopropanol content in 20μL of the standard should not exceed 50%), mix well, and prepare a glycerol standard solution with a concentration of 200μM. Take 0, 1, 2, 5, 10, and

20μL of glycerol standard solution at 200μM each and add them to the standard wells of a 96 well plate. Fill the wells with detection buffer or a detection buffer containing an appropriate amount of isopropanol to 20μL. At this point, the concentrations of the standard curves are 0, 10, 20, 50, 100, and 200μM, respectively.

Note: It is recommended to use a 96 well blackboard for fluorescence detection.

3.2. Add 1-20μL of diluted sample to the 96 well plate sample well, and correspondingly add detection buffer to the sample well to make up to 20μL. Simultaneously set the wells containing only detection buffer or detection buffer containing an appropriate amount of isopropanol as blank controls.

Note: To ensure that the values are within the standard curve range, it is recommended to set multiple dilution factors for the sample simultaneously. Pre experiments can be conducted to determine the approximate concentration of the sample. If the value is not within the standard curve range, please adjust the dilution factor or amount of the sample. The total dilution factor of the sample is recorded as n (including the dilution factor in step 1b). For example, if the dilution factor in 1b is 5 and the sample is further diluted 10 times in this step, the "diluted sample" added is 4μL, then n=5 × 10 × 20/4=250). Attention: For cell or tissue samples prepared with isopropanol, the isopropanol content in 20μL of the sample should not exceed 50% during detection, which is equivalent to the concentration in the final detection system not exceeding 10%.

3.3. (Choose to do)The presence of glycerol-3-phosphate or hydrogen peroxide can interfere with the detection of glycerol. If the sample contains glycerol-3-phosphate or hydrogen peroxide, a sample background control well must be set up at the same time. The specific setting method is the same as step 3b. In step 3d, the glycerol detection working solution prepared normally in step 2b is not added, but a "glycerol detection working solution" without glycerol kinase (using the same volume of detection buffer instead of glycerol kinase) is added.

3.4. Add 80μL of glycerol detection working solution to each well, mix well, and react at 37ºC in the dark for 30 minutes.

Note: If the content of glycerol in the sample is high, the reaction time can be appropriately shortened, such as 15 or 20 minutes; If the low glycerol content in the sample leads to low absorbance or weak fluorescence, the reaction time can be appropriately extended, such as 60 or 90 minutes. At this time, the detection sensitivity will increase, but the high concentration pores of the standard curve will exceed the linear range. For high concentration points that are not within the linear range, they can be omitted in the calculation. Please add "glycerol detection working solution" without adding glycerol kinase (replace glycerol kinase with the same volume of detection buffer) to the sample background control well.

3.5. If absorbance detection is used, measure A570; If fluorescence detection is used, set the excitation wavelength to 560nm and the emission wavelength to 590nm for fluorescence intensity detection.

3.6. Establish a standard curve and calculate the concentration of glycerol in the sample (A). If the background control well signal of the sample is relatively high, the signal value of the sample needs to be subtracted from the background control value. The standard curve of glycerol can refer to Figure 2. The absorbance detection has a good linear relationship in the concentration range of 20-500 μ M, and the fluorescence detection has a good linear relationship in the concentration range of 2-200 μM. The formula for calculating glycerol concentration is as follows: C (μM) = A × n

Note: A is the glycerol concentration (μM) determined based on the standard curve in step 3f; N is the total dilution factor of the sample mentioned in step 3b. The mass concentration (μg/mL) can also be calculated as C × 0.09209 based on the molecular weight of glycerol, which is 92.09.

Note

1. If this kit is used for the detection of cell or tissue samples, isopropanol must be prepared. Cell or tissue samples prepared with isopropanol can be used for the detection of triglycerides, glycerol, free fatty acids, cholesterol, and cholesterol esters. When the sample is an isopropanol extract or lysate of cells or tissues, glycerol detection buffer (hereinafter referred to as detection buffer) should be used to dilute at least twice, so that the isopropanol content in 20μL of the sample is not higher than 50%, which is equivalent to the isopropanol content in the total 100μL detection system not exceeding 10%. At this time, the kit can work normally.
2. To reduce the error caused by the fluorescence background generated by the diluent, the diluent of the standard should be

determined based on the solution used for sample preparation. When the sample is a cell or tissue sample prepared with isopropanol, it can be diluted with the same detection buffer as the isopropanol content in the sample; When the sample is blood or other samples, it is advisable to dilute it with detection buffer.

3. Amplex Red is not very stable in the air. It should be used as soon as possible after opening, and attention should be paid to avoiding light appropriately during use.
4. The reaction products of Amplex Red are highly unstable in the presence of reducing agents, therefore the concentration of dithiothreitol (DTT), β - mercaptoethanol, or similar reducing agents in the final reaction system should be below 10μM.
5. Please ensure that the pH value of the reaction system is between 7-8, otherwise it will affect the stability and fluorescence value of Amplex Red.
6. The detection buffer needs to be completely thawed and equilibrated to room temperature before use, otherwise it will affect the detection results. Other reagents (except for glycerol standard solution (10mM)) should be used on ice.
7. If serum, plasma and other samples are stored at 4ºC, the storage time should not exceed 2 weeks, otherwise it will affect the accuracy of the test results. Usually, serum samples should be stored at -20ºC, with -80ºC being better.
8. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
9. For your safety and health, please wear lab coats and disposable gloves when operating.