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# Glycerine Content Fluorometric Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Fluorescent microplate reader

Catalog Number: AK1044

**Size: 50T/48S ; 100T/96S**

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

| Reagent name | 50T Size | 100T Size | Preservation Condition |
|---|---|---|---|
| Extract Solution | Liquid 60 mL×1 | Liquid 110 mL×1 | 2-8℃ |
| Reagent I | Liquid 5 mL×1 | Liquid 8 mL×1 | 2-8℃ |
| Reagent II | Powder×1 | Powder×1 | -20℃ |
| Reagent III | Powder×1 | Powder×2 | -20℃ |
| Reagent IV | Liquid 0.15 mL×1 | Liquid 0.3 mL×1 | 2-8℃ |
| Reagent V | Powder×2 | Powder×4 | -20℃ |
| Standard | Liquid 1 mL×1 | Liquid 1 mL×1 | |

2-8℃
Black 96-well plate
1 piece
1 piece
-
Sealing film
（Cat#YA0249）
2 sheets
2 sheets
-

Solution preparation:

1.

Reagent II: Before use, add 300μL distilled water to fully dissolve, and unused liquid can be stored at -20℃ for 8 weeks to avoid repeated freezing and thawing.

2.

Reagent III: Dissolve with 15 mL of distilled water one of the bottle before using, and unused liquid can be stored at -20℃ for 8 weeks to avoid repeated freezing and thawing.

3.

Reagent V: Before use, add 0.075 mL of DMSO one of the bottle. After dissolving, aliquot according to the sample volume. Store at room temperature in the dark for immediate use, and store the remaining vials at -20℃ in the dark. It can be stored for 12 weeks. Avoid repeated freezing and thawing.

4.

Working liquid: Before use, the working liquid was prepared according to the sample size in the ratio of Reagent I: Reagent II: Reagent III: Reagent IV : Reagent V : distilled water=60μL: 1μL: 1μL: 2μL: 2μL: 24μL (90μL, 1T). It is recommended to use it up within 2 hours (store at 2-8℃ or on ice).

5.

Standard：1 mmol/mL (1000 μmol/mL) glycerol standard solution. Before use, take 10 μL of the 1000 μmol/mL glycerol standard solution and add 990 μL of Extraction solution. Mix well to prepare a 10 μmol/mL standard solution for use.

Note: Reagent III and Reagent V are freeze-dried reagent, and there may be a large difference or even a small amount of reagent between different bottles. This phenomenon does not affect the use, and the actual quality is the same.

**Product Description：**

Glycerol, has its main chain located in lipids called glycerides. It can serve as an effective marker for liver diseases and is also widely used as a sweetener in the food industry and a humectant in drug formulations. Glycerol is converted into glycerophosphate under the action of glycerol kinase (GK), and glycerol-3-phosphate oxidase (GPO) oxidizes glycerophosphate to produce H2O2. Under the action of enzymes, H2O2 reacts with fluorescent probes to generate highly fluorescent products. The fluorescence intensity at Ex535nm / Em587nm is directly proportional to the content of H2O2. This kit calculates the glycerol content of the sample by determining the generation amount of H2O2.

**Reagents and Equipment Required but Not Provided：**

Microplate Reader (Including fluorescence detection function ), centrifuge, constant temperature incubator/water bath, adjustable pipette, all black 96-well plate (fully black, 1 piece included in the box), sealing film (2 sheets included in the box), DMSO (AR≥98%), mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

**Operation procedure**

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1.

Tissue sample:According to the proportion of tissue mass (g): Extract solution (mL) of 1:5-10 to extract.

It is suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath.

After ice bath homogenization, kept at a boiling water bath for 5min (wrap the sealing film to prevent the cap from bursting). Cool to room temperature and centrifuge at 12000g for 10 min at 4℃, and take the supernatant on ice before testing.

2. Cell/bacteria sample: Collect cells/bacteria into centrifuge tubes, discard the supernatant, add 1 mL of Extract Solution per 10 million cells/bacteria, disrupt the cells by ultrasonic (power 200w, ultrasonic for 3s, 7s interval, totally 3min), then 12000g, 4℃ , centrifuge for 10 min, take the supernatant and put it on ice for testing.

3.

Liquid sample: Take 0.5 mL of the liquid sample, add 0.5 mL of Extraction solution and incubate it in boiling water bath for 5min (wrap the sealing film to prevent the cap from popping off). After cooling to room temperature, centrifuge at 12000g for 10min at 4℃. Take the supernatant and place it on ice for measurement (at this point, the liquid sample has been diluted by 2 times. The ratio of the sample extraction solution can be adjusted according to the experimental results).

Note：If calculated based on protein concentration, after homogenization at ice temperature, centrifuge a portion of the homogenate before boiling it in water bath, and then take the supernatant for protein concentration calculation.

II. Determination procedure：

1. Preheat Fluorescent microplate reader for 30 min, adjust wavelength to Ex530/ Em587nm.

2. Before the experiment, preheat the working liquid to 37℃ for more than 10min.

3. Standard dilution: The 10 μmol/mL (10,000 nmol/mL) glycerol standard solution was diluted with the extraction solution to obtain standard solutions of 50, 25, 12.5, 6.25, 3.125, and 1.5625 nmol/mL for use.

4. Operation Table: (Add the following reagents to each of the black 96-well plate)

Reagent（μL）

Test tube(t)

Standard tube(s)

Blank tube(b)

**Sample**

10

-
-
Standard
-
10
-
Extract Solution
-
-
10
Working liquid
90
90
90

Mix thoroughly, react at 37℃ in the dark for 15 min, measure the fluorescence value at Ex535/ Em587 nm, and record it as RFUt, RFUs, and RFUb. Calculate ΔRFUt = RFUt - RFUb, ΔRFUs = RFUs - RFUb. The standard curve and blank tube only need to be measured 1-2 times.

Pay attention to the sample quantity for each experiment. If the quantity is too large, it can be carried out in batches. Ensure that the fluorescence value measurement is completed within 10 min after the reaction at 37℃ is completed.

III. Calculation：

1． Standard curve: Taking the concentration of each standard solution (nmol/mL) as the x-axis and its corresponding ΔRFUS as the y-axis, draw a standard curve, and bring ΔRFUt into the equation to get x (nmol/mL).

2． Calculation of glycerol content:

(1) Calculate by sample protein concentration

Glycerol content (nmol/mg prot) = x×VS÷(Cpr÷VS)×F = x÷Cpr×F

(2) Calculate by sample mass

Glycerol content (nmol/g mass) = x×VE÷W×F = x÷W×F

(3) Calculate by the number of bacteria or cells

Glycerol content (nmol/106 cell) = x×VE÷N×F = x÷N×F

(4) Calculate by liquid volume

Glycerol content (nmol/mL) =2×x

VS: Sample volume, 0.01mL; VE: Extract solution volume, 1mL; Cpr: Sample protein concentration, mg/mL; W: Sample mass, g; N: The total number of cells or bacteria, count by 106; F: Dilution multiples; 2: The dilution factor in the liquid sample pretreatment process.

**Note:**

1. If the measured value is too small, the sample volume can be increased during the pre-treatment process; if the measured value exceeds the linear range, the sample can be diluted with the extraction solution for

measurement, and it is necessary to simultaneously modify the calculation formula.

2. The fluorescence values (RFU) obtained by different spectrophotometers may vary, which is a normal phenomenon. As long as the reagent preparation and experimental operation are correct, and the standard curve is linear, the detection results will be accurate and reliable.

**Experimental example:**

1.Take 1 mmol/mL glycerol standard solution, dilute to 50, 25, 12.5, 6.25, 3.125, and 1.5625 nmol/mL respectively. Measure the fluorescence values of the blank tube and each standard tube, and obtain RFUb = 103.5. Based on the fluorescence values of each standard tube, establish the standard curve y = 126.36x + 15.246, with R2 = 0.9998.

2. The test results for different types of samples are as follows:

**Sample**

Mass/Volume/Cell count

Dilution multiples

RFUt

ΔRFUt

Glycerol content

Unit

Fetal bovine serum

0.5mL

10

1551.3  1447.8

226.7 nmol/mL

Rat liver

0.1002g

20

1624.9  1521.4

2379.2 nmol/g mass

Peanut

0.1016g

40

2728.8

2625.3

8132.2 nmol/g mass

U251 cell

10×106

1

2344.4  2240.9

1.761 nmol/106 cell