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Glycerine Content Assay Kit

Glycerol Content Assay Kit

Please note: The reagent components and instructions for this product were updated in March 2026. Please take note of this and follow the instructions strictly.

Detection instrument: Spectrophotometer/ Microplate reader

Catalog Number: AK1043-50T-48S

Size: 50T/48S

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	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Powder×1	-20℃
Reagent III	Powder×1	-20℃
Reagent IV	Liquid 1 mL×1	2-8℃
Reagent V	Liquid 3 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃
96-well plate (Cat#YA0501)	1 plate×96wells	-
Sealing film (Cat#YA0249)	2 photos	

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Solution preparation:

1. Reagent II: Before use, add 1.48mL distilled water and dissolve completely. Store unused portion at -20℃ in aliquots for up to 4 weeks. Avoid repeated freeze-thaw cycles.

2. Reagent III: The reagent is placed in a glass bottle. Before use, add 4mL distilled water and dissolve completely. Store unused portion at -20℃ in aliquots for up to 4 weeks. Avoid repeated freeze-thaw cycles. (This reagent is lyophilized; there may be large apparent differences or even very little powder visible, but this does not affect use – the actual mass is the same.)

3. Working Solution: Before use, prepare according to the sample volume using the ratio Reagent II : Reagent III : Reagent IV : Reagent V = 100μL: 200μL: 75μL: 225μL (total 600μL, for 10T). Prepare fresh and use immediately. It is recommended to use within 2 h (store at 2-8℃ or on ice).

4. Standard: 1 mmol/mL glycerol standard solution (1000 μmol/mL)

Product Description:

Glycerol (glycerine) is also known as propanetriol. The glycerol backbone is found in lipids called glycerides. It can serve as an effective marker for liver diseases and is widely used as a sweetener in the food industry and as a humectant in pharmaceutical formulations.

Glycerol is converted to glycerol-3-phosphate by glycerol kinase (GK). Glycerol-3-phosphate oxidase (GPO) oxidizes glycerol-3-phosphate to produce hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine and an aniline analog to form a purple compound with a characteristic absorption peak at 550 nm, which can be used to calculate glycerol content.

Note: It is recommended to perform a preliminary experiment with 2–3 samples expected to show significant differences. If the absorbance of the sample is outside the measurement range, dilute or increase the sample volume accordingly.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, micro glass cuvette/96 well plate, Analytical balance, centrifuge, water bath/constant temperature incubator, 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: Add extraction buffer at a ratio of 1:5-10 based on tissue weight (g) to volume of extraction buffer (mL) (it is recommended to weigh approximately 0.1g of tissue and add 1mL of extraction buffer).

Homogenize on ice, then boil in a water bath for 5 minutes (seal with parafilm to prevent popping).

Cool to room temperature, then centrifuge at 12,000×g for 10 minutes at room temperature. Collect the supernatant and keep on ice.

2.

Bacteria/cells: Based on the number of bacteria/cells (10^6): Use an extraction volume (mL) at a ratio of 5:1 to 10:1 (it is recommended to add 1mL of extraction solution per 5×10^6 bacteria/cells); perform ultrasonic disruption in an ice bath (power 200W, 3s of ultrasonication followed by a 7s interval, for a total of 3min); Then boil in a water bath for 5 minutes (seal with parafilm). Cool to room temperature, centrifuge at

12,000×g for 10 minutes at room temperature, and collect the supernatant on ice.

3.

Serum (plasma): Mix 0.5 mL of liquid sample with 0.5 mL Extract Solution. Boil in a water bath for 5 minutes (seal with parafilm). Cool to room temperature, centrifuge at 12,000×g for 10 minutes at room temperature, and collect the supernatant on ice. (The ratio may be adjusted according to experimental results; modify the calculation formula accordingly.)

Note: For protein concentration-based calculations, take an aliquot of the homogenate before boiling, centrifuge, and use the supernatant for protein determination.

II. Determination operation

1. Preheat the spectrophotometer/microplate reader 30min, adjust the wavelength to 550nm and set zero with distilled water.

2. Preheat Reagent I at 37℃ for 10 minutes before use.

3. Standard dilution: Dilute the 1000μmol/mL glycerol standard with distilled water to obtain the following concentrations: 1.25, 0.625, 0.3125, 0.15625, 0.08, 0.04μmol/mL.

4. Standard Sample Dilution Table

Serial number

Concentration prior to dilution (μmol/mL)

Volume of standard sample (μL)

Volume of distilled water (μL)

Diluted concentration

(μmol/mL)

1

1000

10

990

10

2

10

125

875

1.25

3

1.25

500

500

0.625

4

0.625

500

500
0.3125
5
0.3125
500
500
0.15625
6
0.15625
500
500
0.08
7
0.08
500
500
0.04

Note: Each standard tube requires 20μL of standard solution in the following experiment (please note that the absorbance should not be measured at this stage).

5. Operation table(Add the following reagents sequentially to a 1.5mL Eppendorf tube or 96-well plate):

Reagent name (μL)
Test tube (T)
Standard tube(S)
Blank control (B)
Sample
20
-
-
Standard
-
20
-
Distilled water
-
-
20
Reagent I
120
120

120
Working solution
60
60
60
Mix thoroughly. Incubate at 37℃ for exactly 15 minutes. Measure the absorbance at 550 nm in a micro glass cuvette or 96-well plate (complete measurement within 10 minutes). Record A_test, A_standard, and A_blank. Calculate ΔA_test = A_test – A_blank, ΔA_standard = A_standard – A_blank. The standard curve and blank tube need to be measured only once or twice.

III. Calculation of Glycerol Content:

1. Create standard curve

Plot the standard curve using the standard concentration (x, μmol/mL) and ΔA_standard (y). Then calculate the sample concentration (x) using ΔA_test..

2. Calculation of triglyceride (TG) levels:

(1) Calculated based on the sample protein concentration:

Glycerol content (μmol/g prot) = x × V_sample ÷ (C_protein × V_sample) × F = x ÷ C_protein × F

(2) Calculated based on sample weight:

Glycerol content (μmol/g mass) = x × V_Extract ÷ W × F = x ÷ W × F

(3) Calculated by cell/bacterial count:

Glycerol content (μmol/10^6 cells) = x × V_Extract ÷ N × F = x ÷ N × F

(4) Calculated based on the volume of the liquid sample:

Glycerol content (μmol/mL) = 2 × x

V_sample : volume of sample added, 0.02 mL

V_Extract : volume of Extract Solution added, 1 mL

C_protein : protein concentration of sample, mg/mL

W : sample mass, g

N : number of cells/bacteria (in 10^6)

F : dilution factor

2 : dilution factor for liquid sample pretreatment

Note:

1. If ΔA_test > 2 or A_test > 2, dilute the sample with distilled water and reassay; modify the calculation formula accordingly.

2. Pay attention to the number of samples per experiment. If there are many samples, perform the assay in batches to ensure that absorbance measurement is completed within 10 minutes after the 37℃ reaction.

Experimental example:

1. Take 0.1021 g of rat kidney, add 1 mL Extract Solution, and collect the supernatant. Follow the assay procedure. Measured in a 96-well plate: ΔA_test = A_test – A_blank = 0.758 – 0.050 = 0.708. Standard curve: y = 0.7364x – 0.0406, R^2 = 0.9947. Calculate x = 1.017. Glycerol content by mass = x ÷ W × F = 9.957 μmol/g mass.

2. Take 0.5 mL horse serum, add 0.5 mL Extract Solution, extract as described. Measured in a 96-well plate:

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ΔA_test = 0.123 – 0.050 = 0.073. Using the same standard curve, x = 0.154. Glycerol content by liquid volume = 2 × x = 0.309 μmol/mL