Triglyceride Content Assay Kit (Microanalysis)

NB-64-97541-100T

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#Cat: NB-64-97541-100T Size: 100T

Description:

Triglycerides (TG) are lipid molecules formed from long-chain fatty acids and glycerol. They are not only major components of cell membranes but also serve as important respiratory substrates.

Detection Principle:

Triglycerides (TG) are extracted with isopropanol. After saponification of TG with KOH, they are hydrolyzed to produce glycerol and fatty acids. Glycerol is then oxidized by periodate to generate formaldehyde. In the presence of chloride ions, formaldehyde condenses with acetylacetone to form a yellow compound, which has a characteristic absorption peak at 420 nm. The color intensity is proportional to the TG content.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
Solution A	150 mL x 1 (self-prepared)	4 °C
Solution B	10 mL x 1	4 °C
Solution C	20 mL x 1	4 °C
Solution D	5 mL×1	4 °C, protected from light
Solution E	15 mL×1	4 °C, protected from light
Solution F	15 mL×1	4 °C, protected from light
Solution Standard	1 vial (powder) x 1	Store at −20 °C. Before use, add 1 mL of Solution A to obtain a 1 mg/mL standard triglyceride solution. Store at 4°C protected from light
Preparation of Solution A: Prepare a clean glass vial. Mix n-heptane and isopropanol at a volume ratio of 9:16. Cap tightly and mix thoroughly		

Instructions

I. Required Equipment & Materials:

Visible spectrophotometer/microplate reader, micro glass cuvettes/96-well plates, water bath, adjustable pipettes, double-distilled water, n-heptane, isopropanol, and glass vials.

II. Extraction of TG:

1. Extraction of TG from tissues:

Homogenize the tissue in an ice bath at a ratio of tissue weight (g) to Solution A volume (mL) of 1:5–10 (it is recommended to weigh ~0.1 g tissue and add 1 mL of Solution A). Centrifuge at 8000 × g at 4 °C for 10 min. Collect the supernatant as the TG test solution.

2. Extraction of TG from cells or bacteria:

Collect 4–5 million cells or bacteria into a centrifuge tube and discard the supernatant. Add 1 mL of Solution A and disrupt by ultrasonication for 1 min (20% intensity, 2 s on, 1 s off). Centrifuge at 8000 × g at 4 °C for 10 min. Collect the supernatant as the TG test solution.

3. Serum (plasma) samples: Measure directly

III. Assay Procedure

1. Preheat the visible spectrophotometer/microplate reader for 30 minutes, set the wavelength to 420 nm, and zero with distilled water.
2. Preheat the water bath to 65°C.
3. Perform the following steps in EP tubes

	Blank Tube （μL）	Standard Tube （μL）	Sample Tube （μL）
Distilled Water	120		
1 mg/mL Standard Solution		120	
TG Test Sample			120
Solution A	375	375	375
After adding Solution A, mix thoroughly, then add Solution B			
Solution B	75	75	75
Vortex vigorously for 30 s, let stand for 3–5 min, then vortex vigorously again for 30 s. Repeat this process three times. After phase separation, take 30 μL of the upper layer and transfer it to a new EP tube			

4. Determination of triglyceride content:

	Blank Tube （μL）	Standard Tube （μL）	Sample Tube （μL）
Upper layer solution	30	30	30
Solution C	100	100	100
Solution D	30	30	30
Mix thoroughly, incubate in a 65 °C water bath for 3 min, then cool			
Solution E	100	100	100
Solution F	100	100	100
Mix thoroughly again, incubate in a 65 °C water bath for 15 min, and allow to cool. Transfer 200 μL to a microglass cuvette or a 96-well plate, and measure the absorbance at 420 nm, recorded as A_blank, A_standard, and A_sample.			

IV. Calculation

1. Calculation of triglyceride (TG) content in serum (plasma):

TG content (mg/dL) = C_standard × (A_sample − A_blank) ÷ (A_standard − A_blank) × 100

= 100 × (A_sample −A_blank)÷ (A_standard − A_blank)

C_standard: 1 mg/mL

100: Unit conversion factor (1 dL = 100 mL)

2. Calculation of triglyceride (TG) content in tissues:

(1) Calculated based on protein concentration

TG content (mg/mg prot) = C_standard × V × (A_sample − A_blank) ÷ (A_standard − A_blank) ÷ (C_pr × V) = (A_sample−A_blank) ÷ (A_standard − A_blank) ÷ C_pr

(2) Calculated based on sample weight
TG content (mg/g) = C_standard × V × (A_sample − A_blank) ÷ (A_standard − A_blank) ÷ W
= (A_sample − A_blank) ÷ (A_standard − A_blank) ÷ W

C_standard: 1 mg/mL
C_pr: Protein concentration of the sample (mg/mL)
W: Fresh weight of the sample (g/mL)
V: Volume of Reagent I added (1 mL)

3. Calculation of triglyceride (TG) content in cells or bacteria:
TG content (mg/10^4 cells) = C_standard × (A_sample − A_blank) ÷ (A_standard − A_blank) ÷ cell or bacterial concentration (10^4 cells/L) = (A_sample − A_blank) ÷ (A_standard − A_blank) ÷ cell or bacterial concentration (10^4 cells/L)
C_standard: 1 mg/mL

Precautions

1. The kit contains volatile substances. Gloves and a mask should be worn during the experiment. After opening, reagent bottles should be tightly capped immediately.
2. After adding Solution B , vortex vigorously to ensure thorough extraction of triglycerides from the sample. The shaking amplitude, duration, number of repetitions, and phase separation time should be kept consistent.
3. To ensure reproducibility, the cooling time after each water bath should be kept consistent.
4. If the OD value of the test sample exceeds 1.0, it is recommended to appropriately dilute the sample with Solution A before measurement, and multiply by the corresponding dilution factor in the final calculation. 5. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.