Triglyceride Content Assay Kit (Spectrophotometry)

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
CB0117S-A	100 mL x 1 (self-prepared)	4 °C
CB0117S-B	7 mL x 1	4 °C
CB0117S-C	30 mL x 1	4 °C
CB0117S-D	10 mL×1	4 °C, protected from light
CB0117S-E	30 mL×1	4 °C, protected from light
CB0117S-F	30 mL×1	4 °C, protected from light
CB0117S-Standard	1 vial (powder) x 1	Store at −20 °C. Before use, add 1 mL of CB0117S-A to obtain a 1 mg/mL standard triglyceride solution. Store at 4 °C protected from light.
Preparation of CB0117S-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 CB0117S-A volume (mL) of 1:5–10 (it is recommended to weigh ~0.1 g tissue and add 1 mL of CB0117S-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 CB0117S-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 according to the table below:

	Blank Tube（μL）	Standard Tube（μL）	Sample Tube（μL）
Distilled Water	200		
1 mg/mL Standard Solution		200	
TG Test Sample			200
CB0117M-A	625	625	625
After adding CB0117s-A, mix thoroughly, then add CB0117S-B.			
CB0117M-B	125	125	125
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	150	150	150
CB0117M-C	500	500	500
CB0117M-D	150	150	150
Mix thoroughly, incubate in a 65 °C water bath for 3 min, then cool.			
CB0117M-E	500	500	500
CB0117M-F	500	500	500
Mix thoroughly again, incubate in a 65 °C water bath for 15 min, and allow to cool. Measure the absorbance at 420 nm, recorded as A_blank, A_standard, and A_sample.			

Note: The blank and standard tubes only need to be measured 1–2 times.

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 CB0117S-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 CB0117S-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.