SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Triglyceride(TG)Content Assay Kit(Enzymatic Method)

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Note: The 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: AK1039-100T-96S

Size: 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	Size	Preservation Condition
Extract solution	Required but not provided	-
Reagent I	Liquid 30 mL×1	2-8℃
Reagent II	Powder×1	-20℃
Reagent III	Powder×1	-20℃
Reagent IV	Powder×1	-20℃
Reagent V	Liquid 1.5 mL×1	2-8℃
Reagent VI	Liquid 1 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. Extract solution: Anhydrous ethanol (to be provided by the user); approximately 100mL is required. Store at room temperature. The kit contains a 30mL empty brown bottle, which is intended solely for decanting; please label it with the name of the reagent.

2. Reagent II: Add 1.2mL of distilled water immediately before use and dissolve thoroughly. Store any unused reagent in aliquots at -20°C for up to 4 weeks, avoiding repeated freeze-thaw cycles;

3. Reagent III: The reagent is supplied in a glass vial. Immediately before use, add 4mL of distilled water and dissolve thoroughly. Any unused reagent should be aliquoted and stored at -20°C for up to four weeks; avoid repeated freeze-thaw cycles. (As this is a lyophilised reagent, the volume may appear to vary significantly or even appear very small to the naked eye; this does not affect its performance, and the actual mass remains the same.).

4. Reagent IV: Add 255μL of distilled water immediately before use and dissolve thoroughly. Store any unused reagent in aliquots at 2-8°C for up to 4 weeks, avoiding repeated freeze-thaw cycles.

5. Preparation of Working Solution A and Working Solution B: (Prepare immediately before use; use within 4h; ensure that reagents are added in the correct order)：

Reagent II

Reagent III

Reagent IV

Reagent V

H2O

Reagent VI

Total volume

Working fluid A

0.1mL

0.2mL

0.025mL

0.125mL

0.075mL

0.075mL

Total volume:

0.6 mL, 10 T

Working fluid B

0.1mL

0.2mL

-

0.125mL

0.1mL

0.075mL

Total volume:

0.6 mL, 10 T

Note: Working solution B is used solely for the preparation of the calibration curve.

6. Reference standard: 1mol/L (1000 μmol/L) glycerol standard.

Product Description:

Triglycerides (TG) are widely found in nature and are typically stored in animals and plants in a non-hydrated form. They are one of the primary forms through which animals and humans obtain and store energy, and they also constitute the main component of dietary fat. Within living organisms, the synthesis and breakdown of triglycerides are key processes in energy metabolism. They not only serve as substances for storing and providing energy, but also participate in the construction of cellular structures and the regulation of the properties of biological membranes.

Triglycerides are hydrolysed by lipoprotein lipase (LPL) to form glycerol; glycerol is converted to glycerophosphate by glycerol kinase (GK); glycerophosphate oxidase (GPO) oxidises glycerophosphate to produce hydrogen peroxide; hydrogen peroxide reacts with 4-aminantipyrine and 2, 4-dichlorophenol to form a red product, which exhibits a characteristic absorption peak at 505nm; the triglyceride content can thus be calculated.

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, anhydrous methanol(>98%, AR), 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).

Homogenise the mixture in an ice bath, then centrifuge at 2500g for 10min at room temperature.

Transfer the supernatant to a container and leave at room temperature until analysis.

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 centrifuge at 2500g at room temperature for 10min, and set the supernatant aside at room temperature for analysis.

3.

Serum (plasma): Measure directly. (If the sample is high in fat, dilute it with distilled water before measurement.).

Note: 1. The pre-treatment process inactivates the proteins; therefore, a separate tissue sample must be taken for protein concentration determination.

2. If the supernatant is cloudy after extraction of high-fat samples, or if the reaction mixture is cloudy, the sample supernatant may be diluted with Extract solution prior to analysis; please ensure that the calculation formula is adjusted accordingly.

II. Determination operation

1. Preheat the spectrophotometer/microplate reader 30min, adjust the wavelength to 505nm and set zero with distilled water.

2. Take a sample of the reagent and preheat it at 37℃ for 10min.

3. Dilution of the standard: Dilute the 1000μmol/mL glycerol standard with distilled water to prepare 10, 8, 6, 4, 2 and 1μmol/mL standards for later use (prepare immediately before use and use up on the same day).

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	160	40	8
3	10	120	80	6
4	10	160	240	4
5				

4
200
200
2
6
2
200
200
1

Note: Each standard tube requires 2.5μL 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
2.5
-
-
Standard
-
2.5
-
Distilled water
-
-
2.5
Reagent I
190
190
190
Working fluid A
60
-
-
Working fluid B
-
60

60

Mix thoroughly, incubate at 37°C for exactly 20min, and measure the absorbance at 505nm in a micro- glass cuvette or 96-well plate. Record the values as AT, AS and AB respectively. Calculate ΔAT=AT-AB and ΔAS = AS-AB. Control tube and Blank control and standard curve only need do once or twice.

Note: Once the reaction has stabilised at 37°C, immediately measure the absorbance at 505nm. If the sample volume is too large, the measurement may be carried out in batches, ensuring that the process is completed within 10min.

III. Formula for calculating triglyceride (TG) levels:

1. Create standard curve

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔAs (y, ΔAS), establish a standard curve. According to the standard curve, bring the ΔAT(y) into the formula to calculate the sample concentration (x, μmol/mL).

2. Calculation of triglyceride (TG) levels:

(1) Calculated based on the sample protein concentration:

TG content (μmol/mg prot)= x×VS÷(Cpr×VS)×F =x÷Cpr×F

(2) Calculated based on sample weight:

TG content (μmol/g mass)= x×VE÷W×F=x÷W×F

(3) Calculated by cell/bacterial count:

TG content (μmol/10^6 cell)= x×VE÷N×F=x÷N×F

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

TG content (μmol/mL)= x×F

Vs: Volume of sample added, 0.0025mL;

VT: Volume of extract added, 1mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

N: Cell/bacterial count, expressed in 10^6;

F: Dilution factor.

Note:

1. If the measured value for ΔA is greater than 1, the sample may be diluted appropriately with the Extraction solution before measurement; if the measured value for samples such as cells is low, the sample volume may be increased whilst reducing the volume of Reagent I before measurement, taking care to adjust the calculation formula accordingly.

Experimental example:

1. Take 1000μmol/mL glycerol standard solution and dilute it to 10, 8, 6, 4, 2 and 1μmol/mL. Measure the absorbance of 2.5μL from each standard tube and plot a standard curve based on the absorbance values: y=0.0969x - 0.0919, R^2=0.9935.

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

Sample

Quality/Cell count

Dilution factor A Test ΔA Test TG content unit mouse liver

0.1094g
1
0.267
0.209
28.384 μmol/g mass
Peanuts
0.1038g
4
0.592
0.534
248.911 μmol/g mass
UMR-106 cells
10×106
1
0.069
0.011
0.106 μmol/106 cell human serum
Direct measurement
1
0.120
0.062
1.588 μmol/mL

Note: 1. The above experiment was carried out using a 96-well plate.