

Triglyceride (TG) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: BC0620

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 Solarbio staff in time.

| Reagent Name | Size | Storage |
|--------------|-----------------------|---------|
| Reagent I | Self-supplied reagent | - |
| Reagent II | Liquid 7 mL × 1 | 2-8°C |
| Reagent III | Liquid 20 mL × 1 | 2-8°C |
| Reagent IV | Liquid 10 mL × 1 | 2-8°C |
| Reagent V | Liquid 20 mL × 1 | 2-8°C |
| Reagent VI | Liquid 20 mL × 1 | 2-8°C |
| Standard | Powder × 1 | 2-8°C |

Solution Preparation:

1. Reagent I: Self-supplied reagent, n-heptane and isopropyl alcohol are mixed 1:1 by volume ratio to a glass bottle, which takes about 90mL. Seal and mix well, storage at 2-8°C. The reagent should be prepared just before use.

2. Standard: powder × 1 bottle, add 5 mL of Reagent I before use. 1mg/mL triglyceride standard solution, the unused reagent can be stored at -20°C for 2 weeks, avoid repeated freezing and thawing.

Product Description:

Triglyceride(TG) is a fat molecule formed by long-chain fatty acids and glycerol, which is not only the main component of cell membrane, but also an important respiratory substrate. The TG is extracted with isopropyl alcohol, then hydrolysis to glycerol and fatty acids after saponification of TG by KOH. Glycerol is oxidized by periodic acid to form formaldehyde. Condensation of formaldehyde and acetylacetone to form yellow components in presence of chloride ions. The yellow component has a characteristic absorption at 420 nm and proportional to the TG content.

Technical Indicators:

Minimum Detection limit: 0.0559 mg/mL

Linear Range: 0.0625-1.2 mg/mL

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer, mortar/homogenizer/cell ultrasonic crusher, micro glass cuvette, vortex

oscillators, water bath, adjustable pipette, n-heptane (>98%, AR), isopropyl alcohol (>98%, AR), distilled water, 100 mL empty glass bottle.

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:

Add Reagent I according to the ratio of tissue mass (g) : Reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Reagent I), after ice bath homogenization, centrifuge at 4°C, 8000g for 10min, take supernatant and placed on the ice for test.

2) Bacteria or cell:

The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 2 s, interval 1 s, total time 1min). Centrifuge at 4°C, 8000g for 10min, take supernatant and placed on the ice for test.

3) Serum (plasma) sample:

Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure:

1. Preheat the spectrophotometer/ microplate reader for more than 30 minutes, adjust the wavelength to 420 nm, set zero with distilled water.

2. Preheat water bath to 65°C.

Operation table:

| Reagent name (μL) | Blank tube (A _B) | Standard tube (A _S) | Test tube (A _T) |
|-------------------|------------------------------|---------------------------------|-----------------------------|
| Standard solution | - | 200 | - |
| TG test solution | - | - | 200 |
| Reagent I | 825 | 625 | 625 |
| Reagent II | 125 | 125 | 125 |

Mix thoroughly after adding Reagent I, add Reagent II, shake strongly for 30 s, stand several minutes. After layering, 75 μL of the upper layer solution is taken and put it into a new EP tube.

3. Detect TG content:

| Reagent name (μL) | Blank tube (A _B) | Standard tube (A _S) | Test tube (A _T) |
|--|------------------------------|---------------------------------|-----------------------------|
| Upper layer solution | 75 | 75 | 75 |
| Reagent III | 250 | 250 | 250 |
| Reagent IV | 75 | 75 | 75 |
| Mix thoroughly, water bath at 65°C for 3 minutes. Cool to room temperature | | | |
| Reagent V | 250 | 250 | 250 |
| Reagent VI | 250 | 250 | 250 |

Mix thoroughly, water bath at 65°C for 15 minutes. Cool to room temperature

Take out the EP tubes, colorized at 420 nm after cooling. Record them as A_B , A_S and A_T . The standard curve and blank tube only need to be measured 1-2 times.

III. Calculation:

1 TG content in serum (plasma)

$$TG \text{ (mg/dL)} = C \times (A_T - A_B) \div (A_S - A_B) \times 100 = 100 \times (A_T - A_B) \div (A_S - A_B)$$

2 TG content in tissue or bacteria/cell:

(1) Calculate by sample protein concentration:

$$TG \text{ (mg/mg prot)} = C \times V \times (A_T - A_B) \div (A_S - A_B) \div (C_{pr} \times V) = (A_T - A_B) \div (A_S - A_B) \div C_{pr}$$

(2) Calculate by sample mass:

$$TG \text{ (mg/g)} = C \times V \times (A_T - A_B) \div (A_S - A_B) \div W = (A_T - A_B) \div (A_S - A_B) \div W$$

(3) Calculate by the number of bacteria or cells:

$$TG \text{ (mg / } 10^4 \text{ cell)} = C \times (A_T - A_B) \div (A_S - A_B) \times V \div D = (A_T - A_B) \div (A_S - A_B) \div D$$

100: Unit conversion factor, 1dL =100 mL;

V: The volume of reagent I, 1 mL;

C: Standard concentration, 1 mg/ mL;

C_{pr}: Sample protein concentration (mg/mL);

W: Sample weight(g);

D: Density of bacteria or cell, 10⁴ cell/mL.

Note:

1. There are volatile substances in the kit. Gloves and masks should be worn during the experiment. The reagent bottle cap should be closed in time after opening.

2. After the addition of Reagent II, it is necessary to repeatedly and violently vibrate, so that the triglyceride in the test solution can be fully extracted, and the oscillation amplitude, time, repeated times and waiting for stratification time should be consistent.

3. In order to ensure the repeatability of the test, the cooling time after each water bath should be unified.

4. If the OD value of the test tube is greater than 1, it is recommended to dilute the sample with Reagent I properly before testing, and multiply it by the corresponding dilution multiple during calculation.

Recent Products Citations:

[1] Zhou P, Chang WY, Gong DA, Xia J, Chen W, Huang LY, Liu R, Liu Y, Chen C, Wang K, Tang N, Huang AL. High dietary fructose promotes hepatocellular carcinoma progression by enhancing O-GlcNAcylation via microbiota-derived acetate. Cell Metab. 2023 Nov

7;35(11):1961-1975.e6. doi: 10.1016/j.cmet.2023.09.009. Epub 2023 Oct 4. PMID: 37797623.

[2] Yue C, Li D, Fan S, Tao F, Yu Y, Lu W, Chen Q, Yuan A, Wu J, Zhao G, Dong H, Hu Y. Long-term and liver-selected ginsenoside C-K nanoparticles retard NAFLD progression by restoring lipid

homeostasis. *Biomaterials*. 2023 Oct;301:122291. doi: 10.1016/j.biomaterials.2023.122291. Epub 2023 Aug 20. PMID: 37619263.

[3] Hu C, Xin Z, Sun X, Hu Y, Zhang C, Yan R, Wang Y, Lu M, Huang J, Du X, Xing B, Liu X. Activation of ACLY by SEC63 deploys metabolic reprogramming to facilitate hepatocellular carcinoma metastasis upon endoplasmic reticulum stress. *J Exp Clin Cancer Res*. 2023 May 1;42(1):108. doi: 10.1186/s13046-023-02656-7. PMID: 37122003; PMCID: PMC10150531.

[4] Wang L, Gao T, Li Y, Xie Y, Zeng S, Tai C, Feng Y, Shen P, Wang B. A long-term anti-inflammation markedly alleviated high-fat diet-induced obesity by repeated administrations of overexpressing IL10 human umbilical cord-derived mesenchymal stromal cells. *Stem Cell Res Ther*. 2022 Jun 17;13(1):259. doi: 10.1186/s13287-022-02935-8. PMID: 35715850; PMCID: PMC9204983.

[5] Zhang L, Li XM, Shi XH, Ye K, Fu XL, Wang X, Guo SM, Ma JQ, Xu FF, Sun HM, Li QQ, Zhang WY, Ye LH. Sorafenib triggers ferroptosis via inhibition of HBXIP/SCD axis in hepatocellular carcinoma. *Acta Pharmacol Sin*. 2023 Mar;44(3):622-634. doi: 10.1038/s41401-022-00981-9. Epub 2022 Sep 15. PMID: 36109580; PMCID: PMC9958095.

References:

[1] Fletcher M J. A colorimetric method for estimating serum triglycerides[J]. *Clinica Chimica Acta*, 1968, 22(3): 393-397.

[2] Hercules D M, Sheehan T L. Chemiluminescent determination of serum glycerol and triglycerides[J]. *Analytical chemistry*, 1978, 50(1): 22-25.

Related Products:

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|---------------|---|
| BC1890/BC1895 | Free Cholestenone (FC) Content Assay Kit |
| BC0750/BC0755 | Acetaldehyde Dehydrogenase (ALDH) Activity Assay Kit |
| BC0620/BC0625 | Acetyl CoA carboxylase(ACC) Activity Assay Kit (Enzymatic method) |
| BC1980/BC1985 | Total Cholesterol (TC) Content Assay Kit |