Triglyceride (TG) Content Assay Kit

Cat. No. KITG-078 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Triglyceride (TG) is a lipid molecule formed from long-chain fatty acids and glycerol. It is not only a major component of cell membranes, but also an important respiratory substrate and energy source, and it plays an important role in fatty acid transport. Endogenous triglycerides in serum are transported mainly in the form of very-low-density lipoprotein (VLDL), whereas exogenous triglycerides are transported mainly in the form of chylomicrons (CM). Increased triglyceride content is closely associated with atherosclerotic cardiovascular disease. Triglycerides are saponified and hydrolyzed by KOH to produce glycerol and fatty acids. Periodic acid oxidizes glycerol to formaldehyde. In the presence of ammonium ions, formaldehyde condenses with acetylacetone to form a yellow compound. The product has a characteristic absorption peak at 420 nm, allowing triglyceride content to be quantitatively determined from the change in absorbance.
Description	Method: Visible spectrophotometry. Pack Size: 70 tests / 50 samples. Storage Temperature: 2-8°C. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Quantitative determination of triglyceride (TG) content in bacterial or cell samples, tissues, serum, plasma, culture medium, and other liquid samples by visible spectrophotometry at 420 nm.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer; 1 mL glass cuvette with a 10 mm optical path; mortar or homogenizer; adjustable

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pipettes; benchtop centrifuge; thermostatic water bath; n-heptane; isopropanol; and distilled water. 1. Triglyceride Extraction The sample amount and ratio may be adjusted appropriately according to preliminary experimental results. 1) Bacteria or Cells Collect bacteria or cells in a centrifuge tube by centrifugation. Process the sample at a ratio of bacterial or cell number (10^4) to Reagent 1 volume (mL) of (500-1,000):1. It is recommended to add 1 mL Reagent 1 to 5 million bacteria or cells. Disrupt the bacteria or cells by sonication in an ice bath at 20% power or 200 W, with 2 s sonication and 1 s intervals, for a total of 1 min. Centrifuge at 8,000 × g for 10 min at 4°C. Collect the supernatant for analysis. 2) Tissue Process the sample at a ratio of tissue mass (g) to Reagent 1 volume (mL) of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Reagent 1. Homogenize in an ice bath. Centrifuge at 8,000 × g for 10 min at 4°C. Collect the supernatant for analysis. 3) Serum, Plasma, Culture Medium, and Other Liquid Samples Measure directly or dilute appropriately before measurement. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min, set the wavelength to 420 nm, and zero the instrument with distilled water. 2) Add the following reagents to centrifuge tubes in the stated order: Test Tube: 200 µL TG sample + 625 µL Reagent 1. Standard Tube: 200 µL standard dilution + 625 µL Reagent 1. Blank Tube: 825 µL Reagent 1. Shake thoroughly to mix. Add 125 µL Reagent 2 to each tube. Shake vigorously for 30 s and allow to stand at room temperature for 3-5 min. Repeat this operation three times. Allow the tubes to stand at room temperature until the phases have separated, and collect the upper phase. Transfer 75 µL of the upper phase from each tube to a new tube. Add 250 µL Reagent 3 and 75 µL Reagent 4 to each tube. Mix thoroughly, incubate in a 65°C water bath for 3 min, and cool to room temperature. Add 250 µL Reagent 5 and 250 µL Reagent 6 to each tube. Mix thoroughly, incubate in a 65°C water bath for 15 min, and cool to room temperature. After adding Reagent 2, vigorous shaking is required. Keep the shaking amplitude, shaking time, number of repetitions, and phase-separation waiting time consistent among all tubes. Absorbance Measurement: Transfer the reaction solution to a 1 mL

glass cuvette and measure absorbance at 420 nm. Record the absorbance values as A_test, A_standard, and A_blank. Calculate ΔA_test = A_test - A_blank and ΔA_standard = A_standard - A_blank. The blank tube only needs to be measured 1-2 times. Standard Curve: Use 1.5, 1.0, 0.5, 0.25, 0.125, and 0.0625 mg/mL as the x-axis values and the corresponding ΔA_standard values as the y-axis values. Plot the standard curve and obtain the linear equation y = kx + b. Substitute ΔA_test into the equation to obtain x in mg/mL. 3. Calculation of Triglyceride Content 1) Based on Tissue Protein Concentration: TG content (mg/mg protein) = [x × V_total] / [Cpr × V_total] = x / Cpr. 2) Based on Tissue Sample Mass: TG content (mg/g) = [x × V_total] / W = x / W. 3) Based on Bacterial or Cell Number: TG content (mg/10^4 cells) = [x × V_total] / bacterial or cell number = x / bacterial or cell number. 4) Based on Liquid Sample Volume: TG content (mg/L) = 1,000 × x. Definitions: V_total: total volume of the sample to be tested, 1 mL. Cpr: protein concentration of the sample, mg/mL. W: sample mass, g. Bacterial or cell number: expressed in units of 10^4. 1,000: unit conversion factor, because 1 L = 1,000 mL. Precautions: 1. To ensure reproducible results, after adding Reagent 2, keep the vigorous-shaking amplitude, shaking time, number of repetitions, and phase-separation waiting time consistent. Keep the cooling time after each 65°C water-bath incubation consistent among all assay tubes. 2. If the measured absorbance is outside the linear range of the standard absorbance values, dilute a sample above the highest value appropriately with Reagent 1 and repeat the measurement. For a sample below the lowest value, appropriately increase the sample amount and repeat the measurement. Modify the calculation accordingly. 3. Volatile substances are used during the assay. Perform the measurement in a fume hood and use appropriate protective measures. 4. To ensure accurate results and avoid reagent loss, read the instructions carefully before measurement, confirm that reagents have been stored and prepared correctly, and ensure that all operating steps are understood. Perform a preliminary test using 2-3 samples

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	expected to show substantial differences. Contact technical support promptly if problems arise during the procedure.
Storage	Kit Components and Storage: Reagent 1: liquid, 120 mL × 1 bottle; self-prepared reagent; store at 4°C protected from light. Prepare by mixing n-heptane and isopropanol at a 1:1 volume ratio in a brown light-protective glass bottle. Reagent 2: liquid, 10 mL × 1 bottle; store at 4°C. Reagent 3: liquid, 20 mL × 1 bottle; store at 4°C protected from light. Reagent 4: liquid, 8 mL × 1 bottle; store at 4°C protected from light. Reagent 5: liquid, 20 mL × 1 bottle; store at 4°C protected from light. Reagent 6: liquid, 20 mL × 1 bottle; store at 4°C protected from light. Standard: powder, 1 tube containing 5 mg triglyceride standard; store at 4°C protected from light. Required self-supplied reagents: n-heptane (C_7H_{16}, molecular weight 100.2, CAS 142-82-5) and isopropanol (C_3H_8O, molecular weight 60.01, CAS 67-63-0).
Reconstitution	Reagent Preparation: 1. Reagent 1: Prepare by mixing n-heptane and isopropanol at a 1:1 volume ratio in a brown light-protective glass bottle. 2. Triglyceride Standard Solution: Before use, add 1 mL Reagent 1 to the 5 mg triglyceride standard and dissolve thoroughly to obtain a 5 mg/mL triglyceride standard solution. 3. Standard Dilutions: Dilute the 5 mg/mL triglyceride standard solution with Reagent 1 to prepare 1.5, 1.0, 0.5, 0.25, 0.125, and 0.0625 mg/mL standard dilutions.
Reaction conditions	Measure absorbance at 420 nm. The procedure includes a 65°C water-bath incubation for 3 min followed by a second 65°C water-bath incubation for 15 min, with cooling to room temperature after each incubation.

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