Glycerol Content Assay Kit (Spectrophotometry)

Cat. No. KITG-100 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Glycerol Content Assay Kit (Spectrophotometry) is designed for the determination of glycerol content in tissue samples, bacterial or cell samples, and liquid samples by visible spectrophotometry. Glycerol is catalyzed by glycerol kinase (GK) to form glycerol-1-phosphate (G-1-P). G-1-P is oxidized by glycerol phosphate oxidase (GPO) to generate hydrogen peroxide (H_2O_2). H_2O_2 reacts with 4-aminoantipyrine and other reagents to form a red quinone compound with a characteristic absorption peak at 510 nm. Glycerol content is determined by measuring absorbance at 510 nm. The kit provides reagents for 48 assays.
Description	Glycerol is stored in adipocytes and is one of the final products of triglyceride metabolism. In production and daily life, glycerol can be used as a solvent, lubricant, medicament, and sweetener. Assay Principle Glycerol is catalyzed by glycerol kinase (GK) to produce glycerol-1-phosphate (G-1-P). G-1-P is oxidized by glycerol phosphate oxidase (GPO) to generate hydrogen peroxide (H_2O_2). H_2O_2 reacts with 4-aminoantipyrine and other reagents to form a red quinone compound, which has a characteristic absorption peak at 510 nm. The glycerol content can be obtained by measuring the absorbance at 510 nm. Detection Method Visible spectrophotometry.
Form	Kit
Applications	Determination of glycerol content in tissue samples, bacterial or cell samples, and liquid samples.

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Notes	Size 48 assays. General Notes It is recommended to select 2 samples for a pre-test before the formal experiment in order to understand the condition of the sample batch, become familiar with the experimental procedure, and avoid wasting samples and reagents. This procedure is suitable for most conventional samples. Experimental conditions may be adjusted appropriately according to the actual sample condition. Customized optimization suggestions may be provided for special sample types. Kit Components Extraction Solution: liquid, 60 mL × 1 bottle. Store at 4°C. Reagent I: powder × 1 vial. Store at -20°C. Before use, flick the vial several times to bring the reagent to the bottom, add 4.2 mL distilled water, and shake thoroughly to mix and dissolve. Reagent II: liquid, 25 mL × 1 bottle. Store at 4°C. Reagent III: liquid, 6 mL × 1 bottle. Store at 4°C. Standard: liquid, 1 mL × 1 vial. Store at 4°C. Before use, dilute 10-fold with water to prepare a 4 mM glycerol standard working solution for the assay. Materials Required but Not Supplied Visible spectrophotometer, 1 mL glass cuvette with a 1 cm light path, benchtop centrifuge, adjustable pipette, mortar, and distilled water. Sample Preparation 1. Tissue samples: Weigh approximately 0.1 g tissue into a mortar, add 1 mL Extraction Solution, and homogenize on ice. Centrifuge at 12000 rpm for 10 minutes at 4°C or room temperature, and collect the supernatant for measurement. If the sample amount is increased, extraction may be performed at a tissue weight (g) to Extraction Solution volume (mL) ratio of 1:5-10. 2. Bacterial or cell samples: Collect bacteria or cells in a centrifuge tube, centrifuge, and discard the supernatant. Take approximately 5 million bacteria or cells, add 1 mL Extraction Solution, and disrupt the bacteria or cells by ultrasonication in an ice bath at 200 W, sonicating for 3 seconds at 10-second intervals for 30 cycles. Centrifuge at 12000 rpm for 10 minutes at 4°C, collect the supernatant, and keep it on ice for measurement. If the sample amount is increased, extraction may be performed at a bacterial or cell number (10^4) to Extraction Solution volume (mL) ratio of 500-1000:1. 3. Liquid samples: Clear liquid samples may be measured directly. If a sample is turbid, centrifuge and collect the supernatant for detection.

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Storage	Extraction Solution, Reagent II, Reagent III, and Standard: store at 4°C. Reagent I: store at -20°C.
Reaction conditions	Assay Protocol 1. Preheat the visible spectrophotometer for more than 30 minutes, set the wavelength to 510 nm, and zero the instrument with distilled water. 2. Allow all reagents to thaw and equilibrate to room temperature, 25°C. 3. Add the reagents to EP tubes in the following sequence: Test tube: 20 μL sample, 80 μL Reagent I, 480 μL Reagent II, and 120 μL Reagent III. Standard tube, prepared only once: 20 μL Standard, 80 μL Reagent I, 480 μL Reagent II, and 120 μL Reagent III. Blank tube, prepared only once: 20 μL distilled water, 80 μL Reagent I, 480 μL Reagent II, and 120 μL Reagent III. 4. Mix thoroughly and incubate at room temperature, 25°C, protected from light for 30 minutes. 5. Transfer all liquid from each tube into a 1 mL cuvette and measure the absorbance, A, at 510 nm. Continue reading until the change in A within 2 minutes does not exceed 0.05.
Unit definition	Analysis 1. Calculation based on sample weight: Glycerol (μmol/g weight) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ (W × V1 ÷ V) × D = 4 × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ W × D Glycerol (μg/g weight) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ (W × V1 ÷ V) × D = 368.36 × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ W × D 2. Calculation based on cell number: Glycerol (μmol/10^4 cell) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ (500 × V1 ÷ V) × D = 4 × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ 500 × D Glycerol (μg/10^4 cell) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ (500 × V1 ÷ V) × D = 368.36 × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ 500 × D 3. Calculation of glycerol content in liquid samples: Glycerol (mmol/L) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ V1 × D = 4 × (A_test - A_blank) ÷ (A_standard - A_blank) × D Glycerol (μg/mL) = (C_standard × V2) × (A_test - A_blank) ÷ (A_standard - A_blank) ÷ V1 × D = 368.36 × (A_test - A_blank) ÷ (A_standard - A_blank) × D C_standard: 4 mmol/L =

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	4 μmol/mL = 368.36 μg/mL. V: volume of Extraction Solution, 1 mL. V1: volume of sample added, 0.02 mL. V2: volume of Standard added, 0.02 mL. 500: cell number, 10^4. D: dilution factor; use 1 when the sample is not diluted. W: sample weight, g.
Usage	For Research Use Only. Not for Use in Diagnostic or Therapeutic Procedures.

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