Glycerol Assay Kit (Colorimetric)

Cat. No. KITG-053 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Glycerol Assay Kit (Colorimetric) is designed for the quantitative measurement of glycerol content in serum (plasma), animal tissue, and cell samples. Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, which is further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator of triglyceride hydrolysis reaction. The assay is based on an enzymatic reaction where glycerol is transformed by enzyme to produce hydrogen peroxide, which is catalyzed by oxidase in the presence of 4-amino-antipyrine and phenol to produce red quinones. The color intensity, measured at 510 nm (range 500-520 nm), is proportional to the glycerol content in the sample. A standard curve is generated using serially diluted standards, and sample concentrations are calculated by interpolation from the standard curve.
Species	Human, Mouse, Rat
Source	Colorimetric
Description	Principle: Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, which is further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator of triglyceride hydrolysis reaction and detection is more important. Glycerol is transformed by enzyme to produce hydrogen peroxide, which is catalyzed by oxidase in the presence of 4-amino-antipyrine and phenol to produce red quinones, the color of which is proportional to the content of glycerol. Intended Use: For research use only. Not for diagnostic or clinical use. Sample Types: Serum

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

(plasma), animal tissue, and cell samples. Detection Range: 0.01 - 1.00 mmol/L. Sensitivity: The analytical sensitivity of the assay is 0.01 mmol/L. Specifications: - Size 1 (48 T): 48 wells, suitable for 32 samples - Size 2 (96 T): 96 wells, suitable for 80 samples Kit Components: Size 1 (48 T): - Reagent 1 (Working Solution): 12.5 mL × 1 vial - Reagent 2 (1.0 mmol/L Standard): 1 mL × 1 vial - Microplate: 48 wells - Plate Sealer: 2 pieces - Sample Layout Sheet: 1 piece Size 2 (96 T): - Reagent 1 (Working Solution): 25 mL × 1 vial - Reagent 2 (1.0 mmol/L Standard): 1 mL × 2 vials - Microplate: 96 wells - Plate Sealer: 2 pieces - Sample Layout Sheet: 1 piece Storage: 2-8°C, 12 months, shading light. Note: The reagents must be stored strictly according to the preservation conditions. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents. Materials Required but Not Provided: Instruments: Water bath, Microplate reader (500-520 nm, optimum wavelength: 510 nm) Reagents: Double distilled water, Isopropanol (AR), PBS (0.01 M, pH 7.4) Reagent Preparation: 1. Equilibrate all the reagents to room temperature before use. 2. The preparation of standard curve: Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 mmol/L. Reference is as follows: Standard Curve Preparation Table: Item : Concentration 0 mmol/L, 1.0 mmol/L standard 0 µL, Double distilled water 200 µL Item : Concentration 0.1 mmol/L, 1.0 mmol/L standard 20 µL, Double distilled water 180 µL Item : Concentration 0.2 mmol/L, 1.0 mmol/L standard 40 µL, Double distilled water 160 µL Item : Concentration 0.4 mmol/L, 1.0 mmol/L standard 80 µL, Double distilled water 120 µL Item : Concentration 0.5 mmol/L, 1.0 mmol/L standard 100 µL, Double distilled water 100 µL Item : Concentration 0.6 mmol/L, 1.0 mmol/L standard 120 µL, Double distilled water 80 µL Item : Concentration 0.8 mmol/L, 1.0 mmol/L standard 160 µL, Double distilled water 40 µL Item : Concentration 1.0 mmol/L, 1.0 mmol/L standard 200 µL, Double distilled water 0 µL Sample Preparation: 1. Serum and plasma:

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a period. 2. Tissue sample: - Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). - Wash tissue in cold PBS (0.01 M, pH 7.4). - Homogenize 20 mg tissue in 180 µL isopropanol with a dounce homogenizer at 4°C. - Then inactivated by water bath at 70°C for 10 min, centrifuge at 10000 × g for 10 min, take the supernatant for detection. 3. Cells: - Harvest the number of cells needed for each assay (initial recommendation 2 × 10^6 cells). - Wash cells with PBS (0.01 M, pH 7.4). - Homogenize 2 × 10^6 cells in 100 µL isopropanol with sonicate or mechanical homogenate at 4°C. - Then inactivated by water bath at 70°C for 10 min, centrifuge at 10000 × g for 10 min, take the supernatant for detection. Dilution of Sample: The recommended dilution factor for different samples is as follows (for reference only): - 10% Mouse liver tissue homogenate: Dilution factor 2 - 10% Mouse kidney tissue homogenate: Dilution factor 1 - 10% Mouse spleen tissue homogenate: Dilution factor 1 - Cell: Dilution factor 1 Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor. The Key Points of the Assay: 1. Touch the bottom of the plate when adding standards and samples. 2. There should be no bubbles in the wells of the microplate when measuring the OD value. Operating Steps: 1. Standard well: Add 10 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 10 µL of sample to the corresponding wells. 2. Add 250 µL of working solution to each well. 3. Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min. 4. Measure the OD value of sample well at 510 nm with microplate reader. Calculation: The Standard Curve: 1. Average the duplicate reading for each standard. 2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absoluted OD value. 3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL). The Sample: 1. Serum (plasma) sample: Glycerol content (mmol/L) = (ΔA510 - b) ÷ a × f 2. Tissue sample: Glycerol content (mmol/kg wet weight) = (ΔA510 - b) ÷ a × V ÷ m × f 3. Cell

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

sample: Glycerol content (μmol/10^6 cells) = (ΔA510 - b) ÷ a × V ÷ N × f Note: ΔA510: OD Sample - OD Blank (OD Blank is the OD value when the standard concentration is 0). a: Slope of the standard curve. b: Intercept of the standard curve. V: The volume of isopropanol, mL. m: The weight of sample, g. N: The number of cells, 10^6. f: Dilution factor of sample before test. Performance Characteristics: Intra-assay Precision: Three human serum samples were assayed in replicates of 20 to determine precision within an assay. - Sample 1: Mean 0.25 mmol/L, %CV 4.2 - Sample 2: Mean 0.64 mmol/L, %CV 3.9 - Sample 3: Mean 0.82 mmol/L, %CV 3.9 Inter-assay Precision: Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays. - Sample 1: Mean 0.25 mmol/L, %CV 8.2 - Sample 2: Mean 0.64 mmol/L, %CV 7.8 - Sample 3: Mean 0.82 mmol/L, %CV 8.0 Sensitivity: The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration. Standard Curve Reference Data: Concentration (mmol/L): 0.00, 0.10, 0.20, 0.40, 0.50, 0.60, 0.80, 1.00 Average OD: 0.072, 0.122, 0.175, 0.293, 0.340, 0.394, 0.505, 0.601 Absoluted OD: 0.000, 0.050, 0.103, 0.221, 0.269, 0.323, 0.433, 0.530 Cautions: 1. This assay kit is for Research Use Only. We will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purpose. 2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments. 3. Protection methods must be taken by wearing lab coat and latex gloves. 4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. 5. It is recommended to take a pre-test if your sample is not listed in the instruction book. 6. The experimental results are closely related to the situation of reagents, operations, environment and so on. The quality of the kits is guaranteed only, and the sample consumption caused by using the assay kits is not the responsibility. It is better to calculate the possible usage of sample and reserve

	sufficient samples before experiments.
Form	Colorimetric Assay Kit (96-well/48-well microplate format)
Applications	Quantitative measurement of glycerol content in serum (plasma), animal tissue, and cell samples. Suitable for research use in studies related to triglyceride metabolism, lipid metabolism, and energy metabolism.
Notes	1. This assay kit is for Research Use Only. We will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purpose. 2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments. 3. Protection methods must be taken by wearing lab coat and latex gloves. 4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. 5. It is recommended to take a pre-test if your sample is not listed in the instruction book. 6. The experimental results are closely related to the situation of reagents, operations, environment and so on. The quality of the kits is guaranteed only, and the sample consumption caused by using the assay kits is not the responsibility. It is better to calculate the possible usage of sample and reserve sufficient samples before experiments.
Storage	2-8°C, 12 months, protected from light.
Concentration	Standard concentration: 1.0 mmol/L. Detection range: 0.01 - 1.00 mmol/L.
Shipping	Shipped at ambient temperature. Upon receipt, store at 2-8°C, protected from light.
Reconstitution	Reagent 1 (Working Solution): Ready to use. Reagent 2 (Standard): Dilute with double distilled water to prepare standard curve (0.0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 mmol/L).

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Usage	For research use only. Not for diagnostic or clinical use.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA