Datasheet for ABIN2345061

Free Glycerol Assay Kit (Fluorometric)

Go to Product page

Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Sensitivity:	1 µM
Characteristics:	Free Glycerol Assay Kit measures free, endogenous glycerol by a coupled enzymatic reaction system. The glycerol is phosphorylated and oxidized, producing hydrogen peroxide which reacts with the kit's Fluorometric Probe (Ex. 530-560 nm/Em. 585-595 nm). The Free Glycerol Assay Kit is a simple, fluorometric assay that quantitatively measures the amount of glycerol in plasma or serum in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, glycerol standards and unknown samples. The kit contains a glycerol standard and has a detection sensitivity limit of ~1 µM (0.009 mg/dL).
Components:	1. Glycerol Standard (1 M) : One 200 µL vial of a 1 M glycerol solution. 2. 10X Assay Buffer : One 1.5 mL vial. 3. 5X Enzyme Mixture : Four 525 µL vials. 4. 200X Fluorometric Probe : One 55 µL amber vial. 2
Material not included:	1. Standard 96-well fluorescence black microtiter plate 2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 4. Multichannel micropipette reservoir

Product Details

5. Fluorescence microplate reader capable of reading excitation in the 530-560 nm range and emission in the 585-595 nm range.

Target Details

Background:	Glycerol is the backbone of Triglycerides (TAG). Triglycerides are a type of lipid in the blood, serving as an energy source and playing a key role in metabolism. Triglycerides are the digestive end product of breaking down dietary fats. Any extra carbohydrates and fats that are not immediately used are chemically converted into triglycerides. In the intestines, secreted enzyme lipases hydrolyse the triglyceride ester bond, yielding glycerol and free fatty acids in a process called lipolysis. Enterocytes then absorb and repackage the fragments with cholesterol to form chylomicrons, a major lipoprotein transport particle. In the liver, hepatic lipases also break down triglycerides to assemble another lipoprotein particle (VLDL) from triglycerides, cholesterol, and apolipoproteins.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quantitatively measures the amount of glycerol in plasma or serum in a 96-well microtiter plate format • Kits provide sufficient reagents to perform up to 100 assays, including blanks, glycerol standards and unknown samples
Reagent Preparation:	• Glycerol Standard, 10X Assay Buffer, and 5X Enzyme Mixture should be thawed/maintained at 4 °C during assay preparation. All are stable for 1 week at 4 °C. For longer term storage, each should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws. • 200X Fluorometric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws.
Sample Preparation:	3 • Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4 °C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80 °C for storage. Plasma may need to be diluted (up to 1:4 in PBS) before assaying. Normal human plasma has a glycerol concentration in the range of 0.12-0.61 mg/dL. • Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately

Application Details

	or frozen at -80 °C for storage. Serum may need to be diluted (up to 1:4 in PBS) before assaying. Normal human serum has a glycerol concentration in the range of 0.4-1.2 mg/dL.
Assay Procedure:	Each glycerol standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed. 1. Add 10 µL of the diluted glycerol standards or samples to the 96-well fluorescence microtiter plate. 2. Maintain all components/mixtures at 4 °C. According to Table 2 (below), prepare the desired volume of Reaction Mixture (based on the # of tests) in the following sequence: a. In a tube, add the appropriate volume of deionized water. b. To the water add the corresponding volume of 10X Assay Buffer. Mix well. c. Next, add the corresponding volume of 5X Enzyme Mixture. d. Finally, add the corresponding volume of 200X Fluorometric Probe. Mix well and immediately use. Note: Reaction Mixture will appear slightly pink in color. This is normal background and should be subtracted from all absorbance values. Deionized 10X Assay 5X Enzyme 200X Total Volume # of Tests in Water (mL) Buffer (mL) Mixture (mL) Fluorometric of Reaction 96-well Plate Probe (µL) Mixture (mL) (90 µL/test) 5.950 1 2 50 9 100 2.975 0.5 1 25 4.5 50 1.190 0.2 0.4 10 1.8 20 Table 3. Preparation of Reaction Mixture 4. Transfer 90 µL of the above Reaction Mixture to each well (already containing 10 µL of glycerol standard or sample). 5. Cover the plate wells to protect the reaction from light. 6. Incubate at room temperature for 15 minutes on an orbital shaker. 7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-560 nm range and for emission in the 585-595 nm range. 4 8. Calculate the concentration of glycerol within samples by comparing the sample fluorescence to the standard curve. Negative controls (without glycerol) should be subtracted.
Restrictions:	For Research Use only

Handling

Storage:	-80 °C
Storage Comment:	Store entire kit at -80°C. Avoid multiple freeze/thaws by aliquoting. The Fluorometric Probe is light sensitive and should be maintained in amber tubes.