Free Glycerol Assay Kit (Colorimetric)

Free Glycerol Assay Kit (Colorimetric) is a detection kit for the quantification of Free Glycerol in serum and plasma.

Catalog number: ARG81385

Package: 100 assays

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INTRODUCTION

Glycerol (also called glycerine or glycerin) is a simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in those lipids known as glycerides. Due to having antimicrobial and antiviral properties it is widely used in FDA approved wound and burn treatments. It can be used as an effective marker to measure liver disease. It is also widely used as a sweetener in the food industry and as a humectant in pharmaceutical formulations. Owing to the presence of three hydroxyl groups, glycerol is miscible with water and is hygroscopic in nature.

Glycerol is generally obtained from plant and animal sources where it occurs in triglycerides, esters of glycerol with long-chain carboxylic acids. The hydrolysis, saponification, or transesterification of these triglycerides produces glycerol as well as the fatty acid derivative. [Provide by Wikipedia: glycerol]

PRINCIPLE OF THE ASSAY

This Free Glycerol Assay Kit (Colorimetric) employs a convenient colorimetric method for the detection of free Glycerol from plasma and serum sample. First prepare the desired volume of Reaction Mixture. Transfer the Reaction Mixture and add sample or Standards to the microplate. Incubation with cover to protect from light. The intensity of the color is measured at a wavelength of 540-570 nm. The amount of free glycerol in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at -80°C. Avoid multiple freeze/thaw cycles. Use the kit before expiration date.

Component	Quantity	Storage information
Standards (Glycerol, 1M)	200 µL vial	-80°C
200X Colorimetric Probe	55 µL amber vial	-80°C (protect from light)
5X Enzyme Mixture	4 X 525 µL vials	-80°C
10X Assay Buffer	1.5 mL vial	-80°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 540-570 nm
- Deionized or Distilled water
- 96 well microtiter plate
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -80°C at all times.
- Standards, 10X Assay Buffer, and 5X Enzyme Mixture should be thawed and 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 Colorimetric Probe should be thawed and maintained at room temperature (20-25°C) during assay preparation. Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.
- The Colorimetric Probe is light sensitive and should be maintained in amber tubes.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Sample should be tested immediately or frozen at -80°C for storage. Plasma does not need to be diluted before assaying.

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. Sample should be tested immediately or frozen at -80°C for storage. Serum does not need to be diluted before assaying.

REAGENT PREPARATION

- **Standards:** To prepare the glycerol standards, first perform a 1:100 dilution of the stock 1 M Glycerol Standard in deionized water (E.g. Add 10 µL of 1 M Glycerol Standards to 990 µL deionized water). Use this working Standard (10 mM) to prepare Standards in the concentration range of 0-400 µM.

Dilute Standards as according to the table below:

Standard tube	Final Glycerol conc. (µM)	Deionized Water (µL)	Volume of working standard (µL)
S1	400	480	20 of working standard
S2	200	250	250 of S1
S3	100	250	250 of S2
S4	50	250	250 of S3
S5	25	250	250 of S4
S6	12.5	250	250 of S5
S7	6.25	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Standards and samples should be assayed in duplicates or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **10 µL** of **sample** and serial **diluted Standards** into 96-well microplate.
2. Maintain all components and mixture at **4˚C.** Prepare the desired volume of **Reaction Mixture** with the table below.

Deionized water (mL)	10X Assay Buffer (mL)	5X Enzyme Mixture (mL)	200X Colorimetric Probe (µL)	Total Volume of Reaction Mixture (mL)	# Test in 96-well plate (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

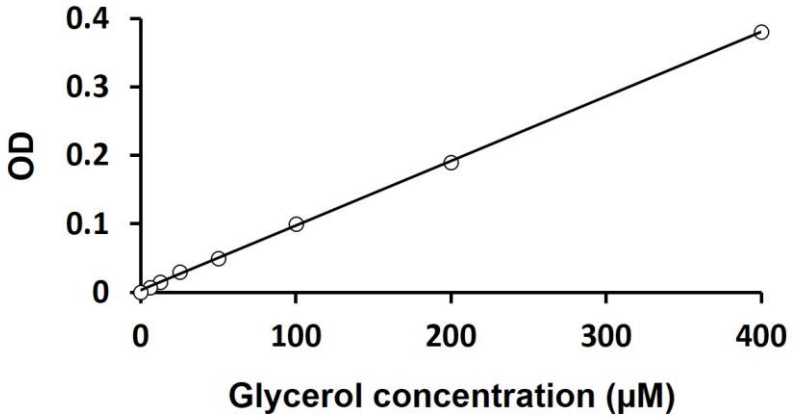
3. Transfer **90 µL** of the above **Reaction Mixture** to each well.
4. Cover the plate to protect from light and incubate at **room temperature** for **15 minutes** on a microplate shaker.
5. Read the OD with a microplate reader at **540-570 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve. Blank (without glycerol) should be subtracted.
4. Automated method: The results in the IFU have been calculated automatically using a linear curve fit.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. Normal human plasma has a glycerol concentration in the range of 0.12-0.61 mg/dL.
7. Normal human serum has a glycerol concentration in the range of 0.4-1.2 mg/dL.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Free Glycerol Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %