Product Manual

Free Glycerol Assay Kit (Colorimetric)

Catalog Number

STA-398 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

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.

Cell Biolabs' 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 Colorimetric Probe (absorbance maxima of 570 nm).

The Free Glycerol Assay Kit is a simple, colorimetric 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 ~5 µM (0.046 mg/dL).

Related Products

1. STA-241: Human Low Density Lipoprotein
2. STA-243: Human High Density Lipoprotein
3. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
4. STA-368: Human ApoB-100 ELISA Kit
5. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
6. STA-375: Uric Acid/Uricase Assay Kit
7. STA-378: Creatinine Assay Kit
8. STA-390: Total Cholesterol Assay Kit
9. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
10. STA-394: HDL Cholesterol Assay Kit
11. STA-399: Free Glycerol Assay Kit (Fluorometric)

Kit Components

1. Glycerol Standard (1 M) (Part No. 239801): One 200 µL vial of a 1 M glycerol solution.
2. 10X Assay Buffer (Part No. 239802): One 1.5 mL vial.
3. 5X Enzyme Mixture (Part No. 239803): Four 525 µL vials.
4. 200X Colorimetric Probe (Part No. 239804): One 55 µL amber vial.

Materials Not Supplied

1. 96-well 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
5. Microplate reader capable of reading at 570 nm

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- Glycerol Standard, 10X Assay Buffer, and 5X Enzyme Mixture should be thawed/maintained at 4ºC during assay preparation. All are stable for 1 week at 4ºC. For longer term storage, each should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 200X Colorimetric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of Glycerol Standard

- To prepare the glycerol standards, first perform a 1:100 dilution of the stock 1 M Glycerol Standard in deionized water. Prepare only enough for immediate use (e.g. Add 10 µL of 1 M Glycerol Standard to 990 µL deionized water). This solution has a concentration of 10 mM. Use this 10 mM glycerol solution to prepare standards in the concentration range of 0 µM – 400 µM by further diluting in deionized water (e.g. Add 20 µL of 10 mM glycerol solution to 480 µL deionized water - see Table 1 below). Glycerol diluted solutions and standards should be prepared fresh.

Standard Tubes	10 mM Glycerol Standard (µL)	Deionized Water (µL)	Final Glycerol Standard (µM)	Final Glycerol Standard (mg/dL)
1	20	480	400	3.68
2	250 of Tube #1	250	200	1.84
3	250 of Tube #2	250	100	0.92
4	250 of Tube #3	250	50	0.46
5	250 of Tube #4	250	25	0.23
6	250 of Tube #5	250	12.5	0.12
7	250 of Tube #6	250	6.25	0.06
8	0	250	0	0

Table 1. Preparation of Glycerol Standards

Preparation of Samples

- 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 does not need to be diluted 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 or frozen at -80°C for storage. Serum does not need to be diluted before assaying. Normal human serum has a glycerol concentration in the range of 0.4-1.2 mg/dL.

Assay Protocol

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 microtiter plate.
2. **Maintain all components/mixtures at 4ºC.** 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 Colorimetric 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 Water (mL)	10X Assay Buffer (mL)	5X Enzyme Mixture (mL)	200X Colorimetric Probe (µL)	Total Volume of Reaction Mixture (mL)	**# of Tests 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**

Table 2. Preparation of Reaction Mixture

3. Transfer 90 µL of the above Reaction Mixture to each well (already containing 10 µL of glycerol standard or sample).
4. Cover the plate wells to protect the reaction from light.
5. Incubate at room temperature for 15 minutes on an orbital shaker.
6. Read absorbance in the 540-570 nm range on a microplate reader.

7. Calculate the concentration of glycerol within samples by comparing the sample absorbance to the standard curve. Negative controls (without glycerol) should be subtracted.

Example of Results

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

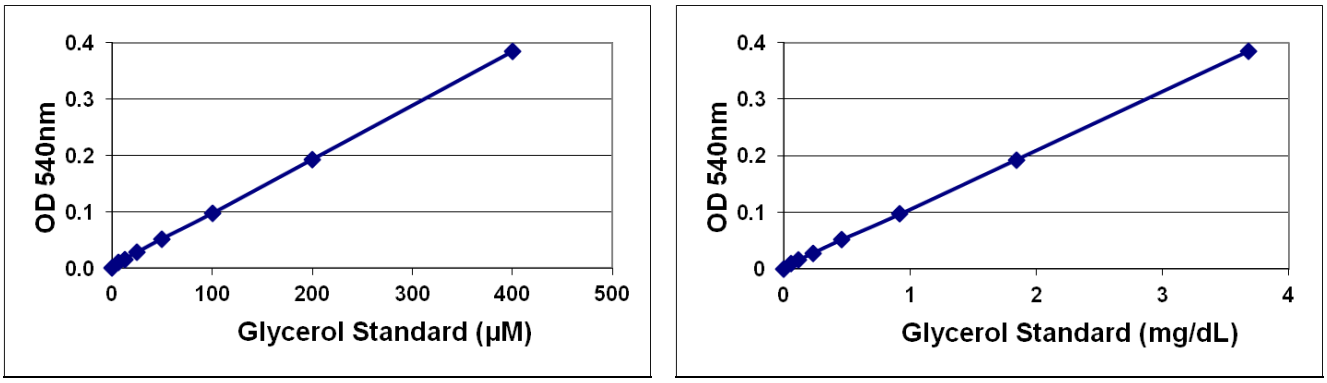

Figure 1: Free Glycerol Assay Standard Curve. Glycerol standard curve was performed according to the Assay Protocol, data plotted in µM (left) and mg/dL (right).

References

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3. Ou, C-N and Frawley, V.L. (1985) *Clin. Biochem.* **18(1),** 37-39.
4. Bernert, J.T., Bell, C.J., McGuffey, J.E., and Waymack, P.P. (1992) *J. Chromatogr*. **578**, 1-7.

Recent Product Citations

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2. Porcu, C. et al. (2018). Effects of short-term administration of a glucogenic mixture at mating on feed intake, metabolism, milk yield and reproductive performance of lactating dairy ewes. *Animal Feed Science and Technology*. **243**:10-21. doi:10.1016/j.anifeedsci.2018.06.012.
3. Desarzens, S. et al. (2014). Hsp90 blockers inhibit adipocyte differentiation and fat mass accumulation. *PLoS One.* **9**:e94127.

Warranty

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