Glycerol Colorimetric Assay Kit

Item No. 10010755

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size
10010960	Glycerol Standard	1 vial/400 µl
700732	Standard Diluent Assay Reagent (5X)	1 vial/4 ml
700003	Sodium Phosphate Assay Buffer	1 vial/4 ml
400789	Glycerol Enzyme Mixture	1 vial
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 530-550 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Test tubes
5. 15 ml centrifuge tube

INTRODUCTION

Background

Glycerol is the backbone of triglycerides, the most important storage form of fat. It is an important metabolite in energy metabolism involved in both oxidation and synthetic processes.[1] Under various physiological or pathological conditions, triglycerides are hydrolyzed and release glycerol and free fatty acids into the blood. Unlike the free fatty acids, glycerol cannot by reutilized by adipose tissue. Glycerol is an active precursor of glucose and plays an important role in gluconeogenesis, especially during periods of caloric deprivation.[2] The measurement of circulating levels of glycerol and free fatty acids are considered to reflect lipolysis, and may be useful to evaluate lipolysis under various conditions in clinical studies.[3] The measurement of glycerol is also useful for the correction of glycerol interference in measurements of triglycerides in reference materials and in serum from patients with elevated glycerol concentrations.[4,5] Diagnostically, the measurement of glycerol can be used to identify patients with a deficiency of glycerol kinase, an X-linked inborn error of metabolism characterized by hyperglycerolemia and glyceroluria.[1]

About This Assay

Cayman's Glycerol Assay Kit provides a simple, reproducible, and sensitive tool for assaying glycerol in plasma and serum. The Glycerol Assay measures glycerol by a coupled enzymatic reaction system (Figure 1). Glycerol is phosphorylated by glycerol kinase to produce glycerol-3-phosphate and adenosine-5'-diphosphate (ADP) (eq 1). The glycerol-3-phosphate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2) (eq 2). Peroxidase catalyzes the redox-coupled reaction of H_2O_2 with 4-aminoantipyrine (4-AAP) and N-ethyl-N-(3-sulfopropyl)-*m*-anisidine (ESPA), producing a brilliant purple product (eq 3) with an absorbance maximum at 540 nm.

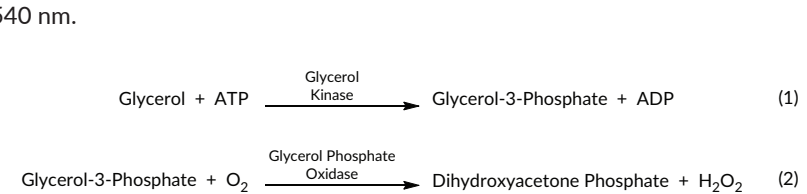

Figure 1. Glycerol assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Glycerol Standard - (Item No. 10010960)**

   The vial contains 400 µl of a 1,000 mg/L solution of Glycerol Standard. It is ready to use to prepare the standard curve.

2. **Standard Diluent Assay Reagent (5X) - (Item No. 700732)**

   The vial contains 4 ml of a (5X) salt solution. Prior to use, dilute the contents of the vial with 16 ml of HPLC-grade water. This Standard Diluent (1X) solution is used to prepare the glycerol standards and may be stored for six months at room temperature until it is ready for use.

3. **Sodium Phosphate Assay Buffer - (Item No. 700003)**

   The vial contains 4 ml of 250 mM sodium phosphate buffer, pH 7.2. Prior to use, dilute the contents of the vial with 16 ml of HPLC-grade water. This diluted buffer (50 mM sodium phosphate, pH 7.2) , Sodium Phosphate Assay Buffer (1X), is used to prepare the glycerol enzyme solution. The Sodium Phosphate Assay Buffer (1X) may be stored for at least six months at room temperature until it is ready for use.

4. **Glycerol Enzyme Mixture - (Item No. 400789)**

   The vial contains a lyophilized enzyme mixture. Reconstitute the vial with 1 ml of HPLC-grade water. Transfer the reconstituted solution to a 15 ml centrifuge tube. Add 14 ml of Sodium Phosphate Assay Buffer (1X) to the reconstituted solution and mix by inversion. *NOTE: A portion of the 14 ml should be used to rinse any residual solution from the vial.* This solution is now ready to use in the assay. If the entire solution is not used at one time, the solution should be stored at 4°C. *NOTE: Do not freeze!* The solution is stable for one month stored at 4°C; a slight pink discoloration may occur but will have no affect on the assay performance.

Sample Preparation

Plasma

Typically, normal human plasma has a glycerol range of 0.12-0.61 mg/dl.[6]

1. Collect blood using either an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

Typically, normal human serum has a glycerol range of 0.4-1.2 mg/dl.[7]

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of glycerol standards and samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 18).

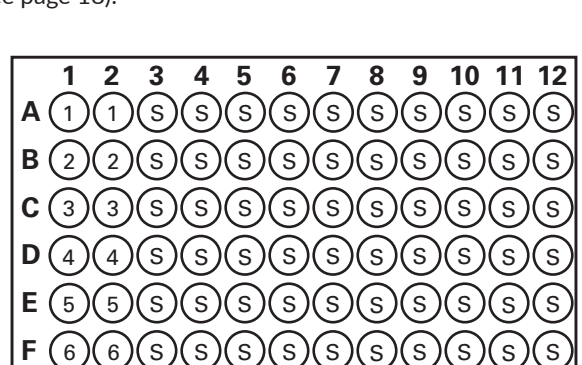

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The final volume of the assay is 200 µl in all wells.
- The incubation is performed at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate.
- Monitor the absorbance at 530-550 nm using a plate reader.

Standard Preparation

Dilute 20 µl of the Glycerol Standard (Item No. 10010960) with 980 µl of Standard Diluent (1X) to obtain a stock solution of 20 mg/L. Take eight clean test tubes and label them 1-8. Add the amount of 20 mg/L glycerol stock solution and Standard Diluent (1X) to each tube as described in Table 1. We recommend that you store these diluted standards for no more than 1-2 hours.

Tube	Glycerol (µl)	Standard Diluent (1X) (µl)	Glycerol Concentration (mg/L)
1	200	0	20
2	150	50	15
3	100	100	10
4	80	120	8
5	60	140	6
6	40	160	4
7	20	180	2
8	0	200	0

Table 1. Preparation of Glycerol Standards

Performing the Assay

1. **Glycerol Standard Wells -** Add 50 µl of Standard (tubes 1-8) per well in the designated wells on the plate (see **Sample Plate Format**, Figure 2, page 9).
2. **Sample Wells -** Add 50 µl of sample (either undiluted plasma or serum) to two or three wells. *NOTE: The amount of sample added to the well should always be 50 µl.*
3. Initiate the reaction by adding 150 µl of the diluted Enzyme Mixture to each well.
4. Carefully shake the microtiter plate for a few seconds to mix. Cover with the plate cover.
5. Incubate the plate for 15 minutes at room temperature.
6. Read the absorbance at 530-550 nm using a plate reader.

ANALYSIS

Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of standard 8 (0 mg/L) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Graph the corrected absorbance values (from step 2 above) of each standard as a function of the final glycerol concentration (mg/L). (see Table 1, page 11) A typical glycerol standard curve is shown in Figure 4, on page 16.
4. Calculate the values of the glycerol samples using the equation obtained from the linear regression of the standard curve by substituting the corrected absorbance values for each sample into the equation.

$$\text{Glycerol (mg/L)} = \left[\frac{\text{(Corrected absorbance) - (y-intercept)}}{\text{Slope}}\right]$$

NOTE: To convert to mg/dl multiply mg/L by 0.1.

Performance Characteristics

Precision:

Intra-assay precision was determined by analyzing 24 replicates of one human heparin plasma control and one human serum control in a single assay.

Matrix Control (mg/L)	%CV
41.3	2.4
5.2	7.0

Table 2. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of one human heparin plasma control and one human serum control in six separate assays on different days.

Matrix Control (mg/L)	%CV
38.2	8.1
5.6	11..4

Table 3. Inter-assay precision

Spike and Recovery

Human EDTA plasma and serum were spiked with different amounts of glycerol and evaluated using the Glycerol Colorimetric Assay Kit. The results are shown below. The error bars represent the standard deviation obtained from multiple dilutions of each sample.

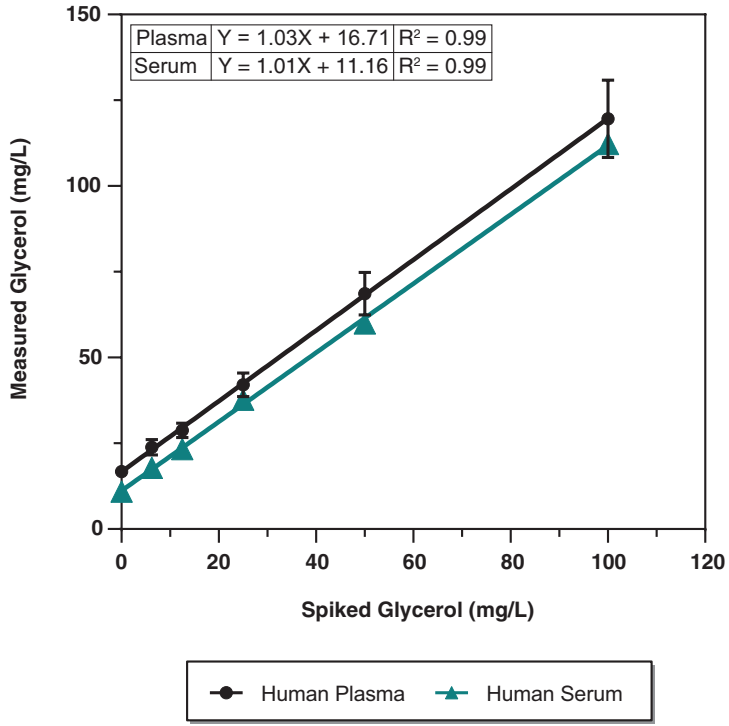

Figure 3. Spike and recovery

Sensitivity:

The lower limit of detection (LLOD) is 0.2 mg/L.

The lower limit of quantification (LLOQ) is 2.0 mg/L.

Representative Glycerol Standard Curve

The standard curve, below, is an example of the data typically provided with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use this data to determine the values of your samples.

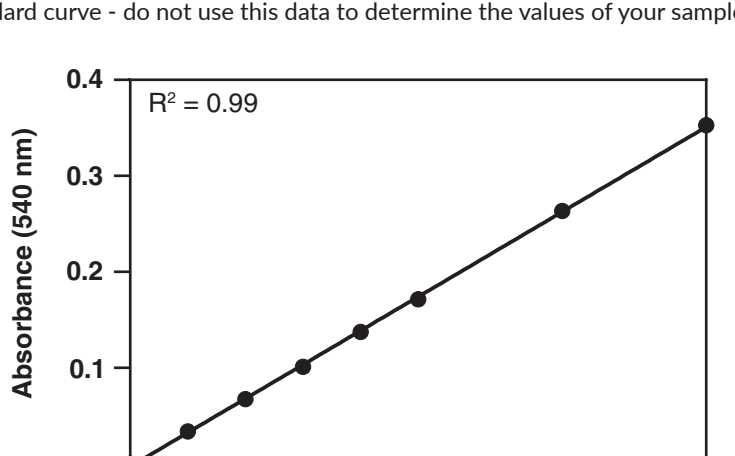

Figure 4. Glycerol standard curve

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No glycerol was detected in the sample	A. Glycerol concentration was too low B. The sample was too dilute	Do not dilute samples and re-assay
Sample absorbance values are above highest point in standard curve	A. Gycerol concentration was too high in the sample B. The sample was too concentrated	Dilute samples with Sodium Phosphate Assay Buffer (1X) and re-assay; *NOTE: Remember to account for the dilution factor when calculating glycerol concentration*

References

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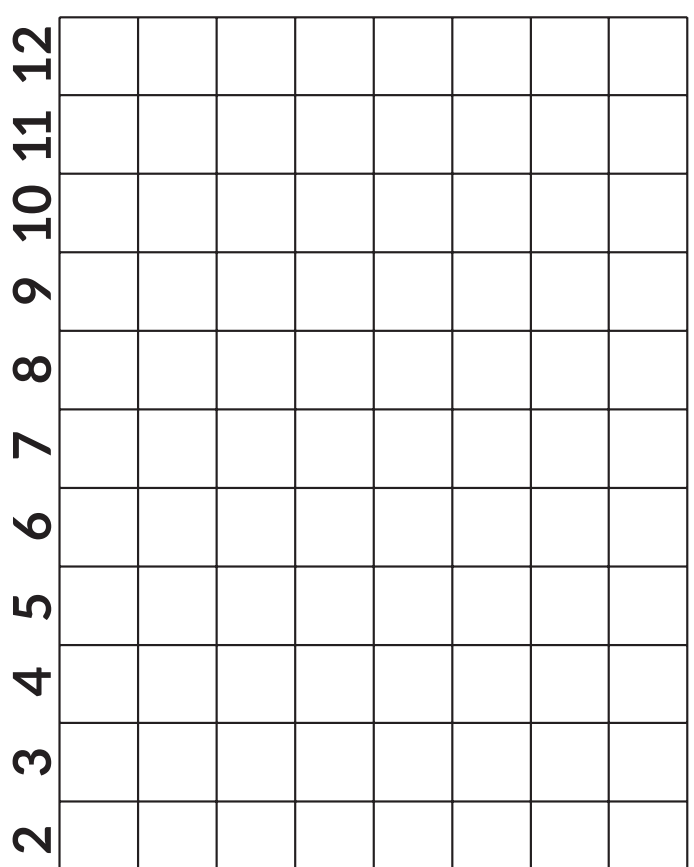

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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