Triglyceride Assay

Colorimetric assay for the quantitative determination of Triglyceride in plasma and serum.

REF CM10010303
Σ 96

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

IBL INTERNATIONAL GMBH
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com
Triglyceride Assay Kit
Catalog No. 10010303
TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
6 About This Assay

PRE-ASSAY PREPARATION
7 Reagent Preparation
8 Sample Preparation

ASSAY PROTOCOL
9 Plate Set Up
11 Standard Preparation
12 Performing the Assay

ANALYSIS
13 Calculations
14 Performance Characteristics

RESOURCES
16 Troubleshooting
17 References
18 Related Products
18 Warranty and Limitation of Remedy
19 Plate Template
20 Notes

GENERAL INFORMATION

Materials Supplied

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10010509</td>
<td>Triglyceride Standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>10010508</td>
<td>Triglyceride Standard Diluent</td>
<td>1 bottle</td>
</tr>
<tr>
<td>10010510</td>
<td>Triglyceride Assay Buffer</td>
<td>1 bottle</td>
</tr>
<tr>
<td>10010511</td>
<td>Triglyceride Enzyme Mixture</td>
<td>1 vial</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Plate</td>
<td>1 plate</td>
</tr>
<tr>
<td>400014</td>
<td>Plate Cover</td>
<td>1 cover</td>
</tr>
</tbody>
</table>

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.
Precautions
Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

If You Have Problems
Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
E-Mail: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

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 as directed 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 repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Test tubes
5. 15 ml centrifuge tube
6. Aluminum foil

INTRODUCTION

Background
Triglycerides are water-insoluble lipids consisting of three fatty acids esterified to a glycerol backbone. Triglycerides are transported in the blood as core constituents of all lipoproteins, but are major components of triglyceride-rich chylomicrons and very low-density lipoproteins (VLDL). A major source of triglycerides is dietary fat. Dietary fats are hydrolyzed in the gut into free fatty acids and mono- and diglycerides and then transported through the intestinal villi. After absorption through the gut, they are resynthesized into new triglycerides and assembled into chylomicrons. Triglycerides are rapidly hydrolyzed in the capillary beds by lipoprotein lipase, releasing glycerol and free fatty acids, which are absorbed by adipose tissue for storage. When required, lipases hydrolyze triglycerides from adipose tissue into fatty acids and glycerol, which enter the blood stream. Fatty acids are oxidized in the mitochondria and peroxisomes to produce energy. Triglycerides play an important role in metabolism by containing more than twice as much energy as carbohydrates and proteins.

The measurement of triglyceride levels, in conjunction with other lipid assays, are useful in the diagnosis of primary and secondary hyperlipoproteinemia, dyslipidemia, and triglyceridemia. Triglyceride concentrations are also useful in the diagnosis and treatment of diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism or various endocrine disorders. The most common method to determine triglyceride concentrations is by enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by either colorimetric or fluorometric measurement of the glycerol released.
About This Assay

Cayman’s Triglyceride Assay provides a simple, reproducible, and sensitive tool for assaying triglycerides in plasma and serum. The Triglyceride Assay uses the enzymatic hydrolysis of the triglycerides by lipase to glycerol and free fatty acids. The glycerol released is subsequently measured by a coupled enzymatic reaction system (Figure 1). The glycerol formed in reaction 1 is phosphorylated to glycerol-3-phosphate in a reaction catalyzed by glycerol kinase (eq 2). The glycerol-3-phosphate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide (eq 3). Peroxidase catalyzes the redox-coupled reaction of H₂O₂ with 4-aminoantipyrine (4-AAP) and N-Ethyl-N-(3-sulfopropyl)-m-anisidine (ESPA), producing a brilliant purple color (eq 4). The absorbance is measured at 540 nm.

Triglycerides → Glycerol + Fatty Acids

Lipoprotein

Glycerol Kinase

Glycerol + ATP → Glycerol-3-Phosphate + ADP

Glycerol Phosphate Oxidase

Glycerol-3-Phosphate + O₂ → Dihydroxyacetone Phosphate + H₂O₂

Peroxidase

2H₂O₂ + 4-AAP + ESPA → Quinoneimine dye + 4H₂O

Figure 1. Triglyceride Assay Scheme

Reagent Preparation

1. Triglyceride Standard - (Catalog No. 10010509)
   Each vial contains a 1,000 mg/dl solution of Triglyceride Standard. It is ready to use as provided to prepare the standard curve. Sufficient Triglyceride Standard is provided to prepare three standard curves.

2. Triglyceride Standard Diluent - (Catalog No. 10010508)
   The vial contains 10 ml of a salt solution. This solution should be thawed and stored at room temperature. This Standard Diluent solution is used to prepare the triglyceride standards and may be stored for six months at room temperature until it is ready for use.

3. Triglyceride Assay Buffer - (Catalog No. 10010510)
   The vial contains 15 ml of 50 mM sodium phosphate buffer, pH 7.2. This solution should be thawed and stored at room temperature. This buffer is used to prepare the triglyceride enzyme solution. The assay buffer may be stored for at least six months at room temperature until it is ready for use.

4. Triglyceride Enzyme Mixture - (Catalog No. 10010511)
   The vial contains a lyophilized enzyme mixture. Reconstitute the contents of the vial with 1 ml of UltraPure water. Transfer the reconstituted solution to a 15 ml centrifuge tube wrapped in aluminum foil. Add 14 ml of the assay buffer 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. Do NOT Freeze! The solution is stable for one month when 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 triglyceride concentrations in the range of 40-160 mg/dl (male) or 35-135 mg/dl (female). 1
1. Collect blood using 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 triglyceride concentrations in the range of 40-160 mg/dl (male) or 35-135 mg/dl (female). 1
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 triglyceride 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 19).

Figure 2. Sample Plate Format

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>D</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>E</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>F</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>G</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>H</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

1-8 = Standards
S = Samples
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 160 µl in all wells.
- The incubation temperature is 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

Take eight clean test tubes and label them 1-8. Add 200 µl of the Triglyceride Standard Diluent (Catalog No. 10010508) to tubes 2-8. Add 400 µl of Triglyceride Standard Diluent (Catalog No. 10010508) to tube 1. Add 100 µl of Triglyceride Standard (Catalog No. 10010509) to tube 1 and mix thoroughly. The concentration of Tube 1 is 200 mg/dl (2.26 mmol/L), from which serial dilutions will be made. Serially dilute the triglycerides by removing 200 µl from tube 1 and adding it to tube 2; mix thoroughly. Next, remove 200 µl from tube 2 and place it into tube 3; mix thoroughly. Repeat this process for tubes 4-7. Tube 8 only has Triglyceride Standard Diluent and is used as the blank. We recommend that you store these diluted standards for no more than one to two hours. See Table 1 below for the triglyceride concentrations of the serial dilutions.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Triglyceride Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>6.25</td>
</tr>
<tr>
<td>7</td>
<td>3.125</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Preparation of Triglyceride Standards
Performing the Assay

1. **Triglyceride Standard Wells** - Add 10 µl of standard (tubes 1-8) per well in the designated wells on the plate (see suggested plate configuration, Figure 2, page 9).

2. **Sample Wells** - Add 10 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 10 µl.

3. Initiate the reaction by adding 150 µl of diluted enzyme buffer solution 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/dl) 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 triglyceride concentration (mg/dl) (see Table 1, page 11). A typical triglyceride standard curve is shown in Figure 3 on page 15.

4. Calculate the values of triglyceride 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.

\[
\text{Triglycerides (mg/dl)} = \left[ \frac{(\text{Corrected absorbance}) - (\text{y-intercept})}{\text{Slope}} \right]
\]
Performance Characteristics

**Precision:**
When a series of sixteen human serum samples were assayed on the same day, the intra-assay coefficient of variation was 1.34%. When a series of sixteen human serum samples were assayed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.17%.

**Assay Range:**
Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-200 mg/dl triglyceride.

**Representative Triglyceride Standard Curve**
The standard curve, presented on page 15, 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 3. Triglyceride standard curve
Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates/triplicates</td>
<td>A. Poor pipetting/technique</td>
<td>A. Carefully tap the side of the plate with your finger to remove bubbles</td>
</tr>
<tr>
<td></td>
<td>B. Bubble in the well(s)</td>
<td>B. Be careful not to splash the contents of the wells</td>
</tr>
<tr>
<td>No triglyceride was detected in the sample</td>
<td>Triglyceride concentration was too low or the sample was too dilute</td>
<td>Do not dilute samples and re-assay</td>
</tr>
<tr>
<td>Sample absorbance values are above highest point in standard curve</td>
<td>Triglyceride concentration was too high in the sample or the sample was too concentrated</td>
<td>Dilute samples with assay buffer and re-assay. NOTE: Remember to account for the dilution factor when calculating the triglyceride concentration.</td>
</tr>
</tbody>
</table>

References

Related Products

- Cholesterol Assay Kit - Cat. No. 10007640
- Cholesterol Cell-Based Detection Assay Kit - Cat. No. 10009779
- ChREBP Transcription Factor Assay Kit - Cat. No. 10006909
- SREBP-2 Cell-Based Translocation Assay Kit - Cat. No. 10009239
- SREBP-2 Transcription Factor Assay Kit - Cat. No. 10007819

Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s exclusive remedy and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Cat.-No.: Kat.-Nr.: No.- Cat.: Cat.-No.: N.º Cat.: N.-Cat.:</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot-No.: Chargen-Bez.: No. Lot: Lot-No.: Lote N.º: Lotto n.:</td>
</tr>
<tr>
<td>E</td>
<td>Use by: Verwendbar bis: Utiliser à: Usado por: Usar até: Da utilizzare entro:</td>
</tr>
<tr>
<td>E</td>
<td>No. of Tests: Kitgröße: Nb. de Tests: No. de Determin.: N.º de Testes: Quantità dei tests:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate/ Konzentrat/ Concentré/ Concentrar/ Concentrado/ Concentrato/ Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized/ Lyophilisat/ Lyophilisé/ Liofilizado/ Liofilizzato/ Λυοφιλιασµένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για Ιν-Βίτρο Διάγνωσης.</td>
</tr>
<tr>
<td>E</td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td>E</td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Να ψηλάσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td>E</td>
<td>Store at: / Lager bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td>E</td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td>E</td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symboles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

---

**IBL AFFILIATES WORLDWIDE**

<table>
<thead>
<tr>
<th>Location</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBL International GmbH</td>
<td>Flughafenstr. 52A, D-22335 Hamburg, Germany Tel.: + 49 (0) 40 532891 -0 Fax: -11 E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a> WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
</tr>
<tr>
<td>IBL Deventer B.V.</td>
<td>Zutphenseweg 55, NL-7418 AH Deventer, The Netherlands Tel.: + 31 570-66 15 15 Fax: -60 73 86 E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a> WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
</tr>
<tr>
<td>IBL - Transatlantic Corp.</td>
<td>288 Wildcat Road, Toronto, Ontario M3J 2N5 Toll free: +1 (866) 645 -6755 Tel.: +1 (416) 645 -1703 Fax: -1704 E-MAIL: <a href="mailto:IBL@IBL-Transatlantic.com">IBL@IBL-Transatlantic.com</a> WEB: <a href="http://www.IBL-Transatlantic.com">http://www.IBL-Transatlantic.com</a></td>
</tr>
</tbody>
</table>

**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.