Triglyceride Colorimetric Assay Kit

Item No. 10010303

Customer Service 800.364.9897 * **Technical Support** 888.526.5351

www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size
10010509	Triglyceride Standard	1 vial/400 µl
700732	Standard Diluent Assay Reagent (5X)	1 vial/12 ml
700003	Sodium Phosphate Assay Buffer	1 vial/4 ml
10010511	Triglyceride Enzyme Mixture	1 vial
400012	96-Well Cover Sheet	1 cover
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate

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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic 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

Email: 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 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
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).[1] 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.[2-4] 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.[5-8]

About This Assay

Cayman's Triglyceride Colorimetric Assay provides a simple, reproducible, and sensitive tool for assaying triglycerides in plasma and serum. The Triglyceride Colorimetric 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 H_2O_2 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.

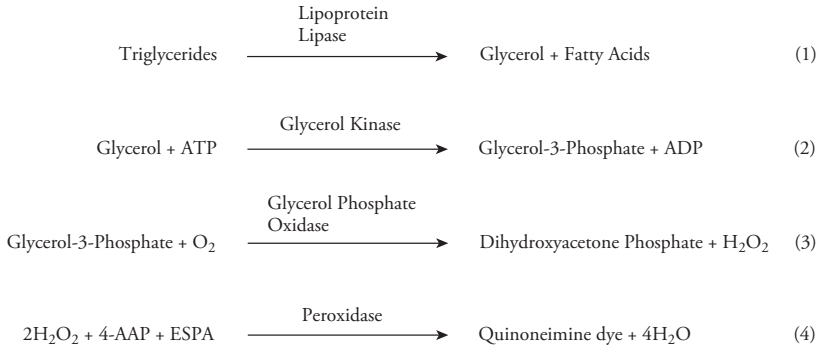


Figure 1. Triglyceride assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. Triglyceride Standard - (Item No. 10010509)

The vial contains 400 µl of 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. Standard Diluent Assay Reagent (5X) - (Item No. 700732)

The vial contains 12 ml of a (5X) salt solution. Prior to use, dilute the contents of the vial with 48 ml of HPLC-grade water. This diluted 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. 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) 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 - (Item No. 10010511)

The vial contains a lyophilized enzyme mixture. Reconstitute the contents of the vial with 1 ml of HPLC-grade water. Transfer the reconstituted solution to a 15 ml centrifuge tube wrapped in aluminum foil. Add 14 ml of the diluted 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).[9]

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).[9]

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.

Cell Lysates

1. Collect cells (~18 x 10^6 cells) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Resuspend the cell pellet in 1-2 ml of cold diluted Standard Diluent.
3. Sonicate the cell suspension 20X at one second bursts.
4. Centrifuge cell suspension at 10,000 x g for 10 minutes at 4°C.
5. Remove the supernatant and store on ice. If not assaying on the same day, freeze at -80°C until use. The sample will be stable for at least one month.
6. Before assaying, further dilute the samples 1:2-1:3 with diluted Standard Diluent.

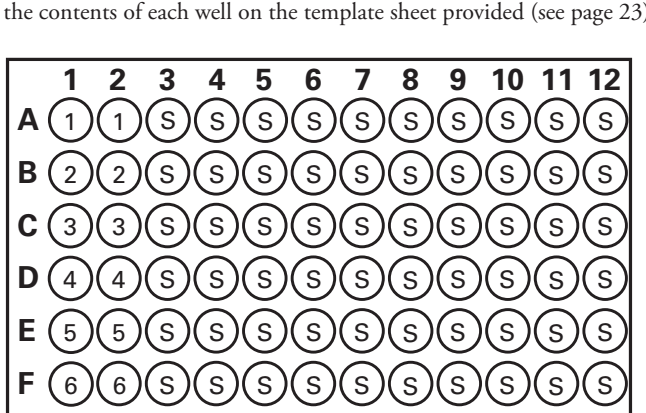
Tissue Homogenates

1. Weigh tissue and then mince into small pieces.
2. Homogenize 350-400 mg of minced tissue in 2 ml of the diluted Standard Diluent containing protease inhibitors of choice (see **Interference** section).
3. Centrifuge at 10,000 x g for 10 minutes at 4°C.
4. Transfer the supernatant to another tube. Store the supernatant on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month while stored at -80°C.
5. Typically, tissue samples require dilutions of at least 1:5 or greater. Dilute the samples using the diluted Standard Dilutent 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 23).



1-8 = Standards
S = Samples

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 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 diluted Standard Diluent (Item No. 700732) to tubes 2-8. Add 400 µl of diluted Standard Diluent to tube 1. Add 100 µl of Triglyceride Standard (Item 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 diluted 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, on page 13, for the triglyceride concentrations of the serial dilutions.

Tube	Triglyceride Concentration (mg/dl)
1	200
2	100
3	50
4	25
5	12.5
6	6.25
7	3.125
8	0

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 10).
2. **Sample Wells** - Add 10 µl of sample 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 17.
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.

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

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 17, 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.

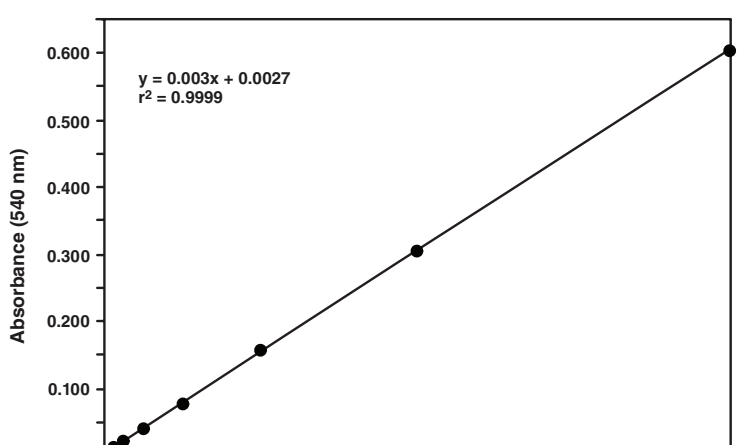


Figure 3. Triglyceride standard curve

Interferences

The following reagents were tested in the assay for interference:

Reagent		Will Interfere (Yes or No)
Buffers	Tris	No
	Borate	No
	HEPES	No
	Phosphate	No
	MES	No
Detergents	Polysorbate 20 (1%)	No
	Triton X-100 (1%)	No
Protease Inhibitors/ Chelators/ Enzymes	EDTA (1 mM)	No
	EGTA (1 mM)	No
	Trypsin (10 µg/ml)	No
	PMSF (200 µM)	Yes
	Leupeptin (10 µg/ml)	No
	Antipain (100 µg/ml)	No
	Chymostatin (10 µg/ml)	No
	BSA (1%)	Yes
Solvents	Ethanol (5%)	Yes
	Methanol (5%)	No
	Dimethylsulfoxide (5%)	No
Others	Sucrose (250 mM)	No
	Glycerol (5%)	Yes

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 triglyceride was detected in the sample	Triglyceride concentration was too low or the sample was too dilute	Do *not* dilute samples and re-assay
Sample absorbance values are above highest point in standard curve	Triglyceride concentration was too high in the sample or the sample was too concentrated	Dilute samples with assay buffer and re-assay; *NOTE: Remember to account for the dilution factor when calculating the triglyceride concentration*

References

1. Cole, T. G.; Klotzsch, S. G.; McNamara, J. R. Measurement of Triglyceride Concentration *in* Handbook of Lipoprotein Testing. N. Rifai, *et. al.*, Ed. AACC Press. Washington DC, 115, (1997).
2. Fredrickson, D.S., Levy, R.I., and Lees, R.S. Fat transport in lipoproteins - an integrated approach to mechanisms and disorders. *New England Journal of Medicine* **276(1)**, 34-42 (1967).
3. Rifai, N., Bachorik, P.S., Albers, J.J. Lipids, Lipoproteins, and Apolipoproteins *in* Tietz Fundamentals of Clinical Chemistry. C.A. Burtis, E.R. Ashwood, Ed. WB Saunders Company, Philadelphia, PA, 462-493 (2001).
4. Wahlefeld, A.W. *in* Methods of Enzymatic Analysis, Vol. 5. H.U. Bergmeyer, Ed. Academic Press, New York, NY, 1831-1835, (1974).
5. Bucolo, G. and David, H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19(5)**, 476-482 (1973).
6. Fossati, P. and Prencipe, L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* **28(10)**, 2077-2080 (1982).
7. McGowan, M.W., Artiss, J.D., Strandbergh, D.R., *et al.* A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29(3)**, 538-542 (1983).
8. Mendez, A.J., Cabeza, C., and Hsia, S.L. A fluorometric method for the determination of triglycerides in nanomolar quantities. *Anal. Biochem.* **156**, 386-389 (1986).
9. Deska-Pagana, K. and Pagana, T.J. *in* Mosby's Diagnostic and Laboratory Test Reference. Seventh Edition, Mosby, St. Louis, 937-938 (2005).

Related Products

Aldehyde Dehydrogenase Activity Assay Kit - Item No. 700800
p-Aminohippuric Acid (PAH) Assay Kit - Item No. 700880
ANGPTL3 (human) EIA Kit - Item No. 580170
ANGPTL6 (human) EIA Kit - Item No. 580190
Calcium Assay Kit - Item No. 700550
Chloride Colorimetric Assay Kit - Item No. 700610
Cholesterol Fluorometric Assay Kit - Item No. 10007640
Cholesterol Cell-Based Detection Assay Kit - Item No. 10009779
ChREBP Transcription Factor Assay Kit - Item No. 10006909
Coenzyme A Assay Kit - Item No. 700440
Creatine Kinase Fluorometric Assay Kit - Item No. 700630
Glucose Colorimetric Assay Kit - Item No. 10009582
Glucose-6-Phosphate Dehydrogenase Activity Assay Kit - Item No. 700300
Glucose-6-Phosphate Fluorometric Assay Kit - Item No. 700750
Glycerol Cell-Based Assay Kit - Item No. 10011725
Glycerol Colorimetric Assay Kit - Item No. 10010755
Glycogen Assay Kit - Item No. 700480
Hemoglobin Colorimetric Assay Kit - Item No. 700540
β-Hydroxybutyrate (Ketone Body) Fluorometric Assay Kit - Item No. 700740
Inulin Fluorometric Assay Kit - Item No. 700770
D-Lactate Assay Kit - Item No. 700520
L-Lactate Assay Kit - Item No. 700510
Lipase Activity Assay Kit - Item No. 700640
Malate Fluorometric Assay Kit - Item No. 700790
Phosphoenolpyruvate Fluorometric Assay Kit - Item No. 700780
Pyruvate Assay Kit - Item No. 700470
Pyruvate Kinase Activity Assay Kit - Item No. 700760
SREBP-2 Cell-Based Translocation Assay Kit - Item No. 10009239
SREBP-2 Transcription Factor Assay Kit - Item No. 10007819
Urea Fluorometric Assay Kit - Item No. 700620
Uric Acid Assay Kit - Item No. 700320

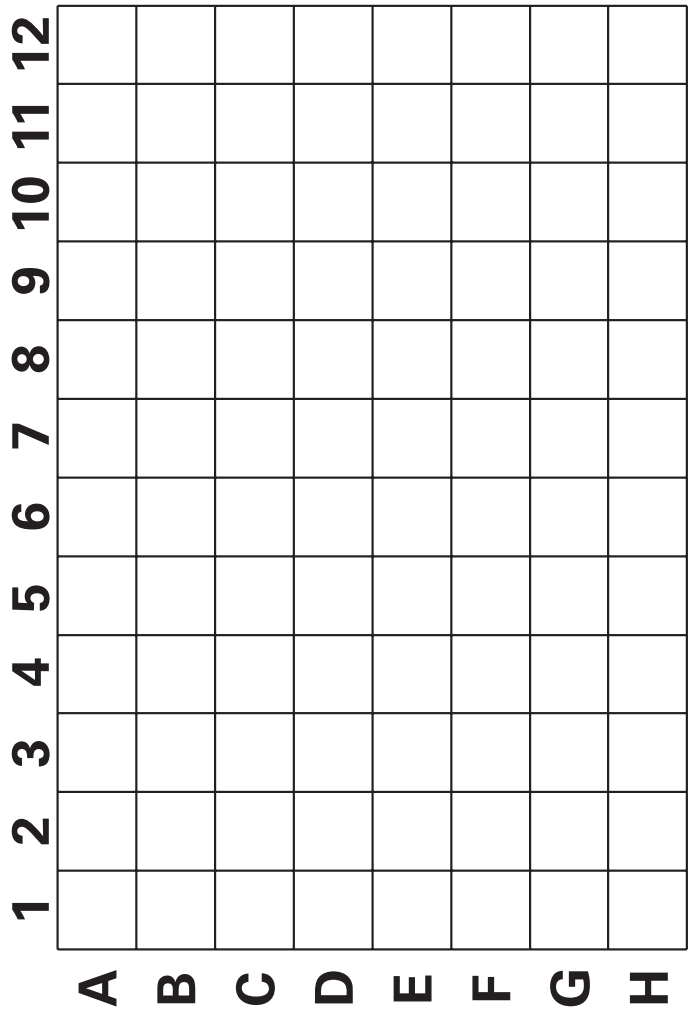
Warranty and Limitation of Remedy

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.



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