
Product Manual

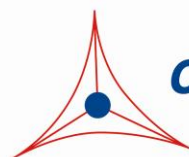
Serum Triglyceride Quantification Kit (Colorimetric)

Catalog Number

STA-396

100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.

Creating Solutions for Life Science Research

Introduction

Triglycerides (TAG) 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' Serum Triglyceride Quantification Kit measures triglyceride concentration in serum, plasma, and lysates by a coupled enzymatic reaction system. First, lipase hydrolyzes the triglyceride ester bond, yielding glycerol. The glycerol is then phosphorylated and oxidized, producing hydrogen peroxide which reacts with the kit's Colorimetric Probe (absorbance maxima of 570 nm).

The Serum Triglyceride Quantification Kit is a simple, colorimetric assay that quantitatively measures the amount of triglyceride in plasma, serum, and lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, triglyceride standards, free glycerol controls and unknown samples. The kit contains a triglyceride standard and has a detection sensitivity limit of ~10 μ M (1 mg/dL).

Related Products

1. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
2. STA-378: Creatinine Assay Kit
3. STA-390: Total Cholesterol Assay Kit
4. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
5. STA-398: Free Glycerol Assay Kit (Colorimetric)

Kit Components (shipped on dry ice)

1. Triglyceride Standard (Part No. 239601): One 200 μ L vial (equivalent to 20,000 mg/dL triglyceride mixture with average MW of 873).
2. 10X Assay Buffer (Part No. 239802): One 1.5 mL vial.
3. 10X Lipase Solution (Part No. 239602): One 1 mL vial.
4. 5X Enzyme Mixture (Part No. 239803): Four 525 μ L vials.
5. 200X Colorimetric Probe (Part No. 239804): One 55 μ L amber vial.

Materials Not Supplied

1. 96-well microtiter plate
2. Microplate reader capable of reading at 570 nm

Storage

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

Preparation of Reagents

- Triglyceride Standard, 10X Assay Buffer, 10X Lipase Solution, and 5X Enzyme Mixture should be thawed/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/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.

Preparation of Triglyceride Standard

- To prepare the triglyceride standards, first perform a 1:100 dilution of the stock Triglyceride Standard in deionized water. Prepare only enough for immediate use (e.g., Add 10 µL of 20,000 mg/dL Triglyceride Standard to 990 µL deionized water). This solution has a concentration of 200 mg/dL. Use this 200 mg/dL triglyceride solution to prepare standards in the concentration range of 0 – 40 mg/dL by further diluting in deionized water (e.g., Add 200 µL of 200 mg/dL triglyceride solution to 800 µL deionized water - see Table 1 below). Triglyceride diluted solutions and standards should be prepared fresh.

Standard Tubes	200 mg/dL Triglyceride Standard (µL)	Deionized Water (µL)	Final Triglyceride Standard (mg/dL)	Final Triglyceride Standard (µM)
1	200	800	40	458
2	250 of Tube #1	250	20	229
3	250 of Tube #2	250	10	114.5
4	250 of Tube #3	250	5	57.25
5	250 of Tube #4	250	2.5	28.63
6	250 of Tube #5	250	1.25	14.31
7	250 of Tube #6	250	0.625	7.156
8	0	250	0	0

Table 1. Preparation of Triglyceride 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 must be diluted before assaying (1:10 to 1:40 in PBS). Normal triglyceride levels in human plasma are considered less than 150 mg/dL; however, very high levels can exceed 500 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 must be diluted before assaying (1:10 to 1:40 in PBS). Normal triglyceride levels in human serum are considered less than 150 mg/dL; however, very high levels can exceed 500 mg/dL.

- Cell Lysates: Collect 10 x 10⁶ cells by centrifugation at 1000 x g for 10 minutes. Discard the supernatant and resuspend in 1 mL of cold PBS containing 1% Triton X-100. Homogenize or sonicate the cell suspension. Centrifuge at 10000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates must be further diluted before assaying (1:5 or greater).
- Tissue Samples: Weigh out 200 mg of tissue and mince into small pieces. Homogenize the minced tissue in 1 mL of cold PBS containing 1% Triton X-100. Centrifuge at 10000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 1 month. Cell lysates must be further diluted before assaying (1:5 or greater).

Assay Protocol

Each triglyceride standard and sample should be assayed in duplicate or triplicate. Additionally, each sample should be tested without lipase to determine free glycerol background. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 10 µL of the diluted triglyceride standards or samples to the 96-well microtiter plate.
2. Maintain all components/mixtures at 4°C. 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. Add the corresponding volume of 5X Enzyme Mixture.
 - d. Next, add the corresponding volume of 10X Lipase Solution.

Note: To determine background free glycerol signal, this step should be skipped (without lipase). Deionized water should be used to make up the volume.

- e. 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)	10X Lipase Solution (mL)	200X Colorimetric Probe (µL)	Total Volume of Reaction Mixture (mL)	# of Tests in 96-well Plate (90 µL/test)
4.950	1	2	1	50	9	100
2.475	0.5	1	0.5	25	4.5	50
0.990	0.2	0.4	0.2	10	1.8	20

Table 2. Preparation of Reaction Mixture

- Transfer 90 μL of the above Reaction Mixture to each well (already containing 10 μL of triglyceride standard or sample).
- Cover the plate wells to protect the reaction from light.
- Incubate at room temperature for 30 minutes on an orbital shaker.
- Read absorbance in the 540-570 nm range on a microplate reader.
- Calculate the concentration of triglyceride within samples by comparing the sample absorbance to the standard curve. Negative controls (without triglyceride) should be subtracted. Absorbance from free glycerol should also be deducted for true triglyceride values.

Example of Results

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

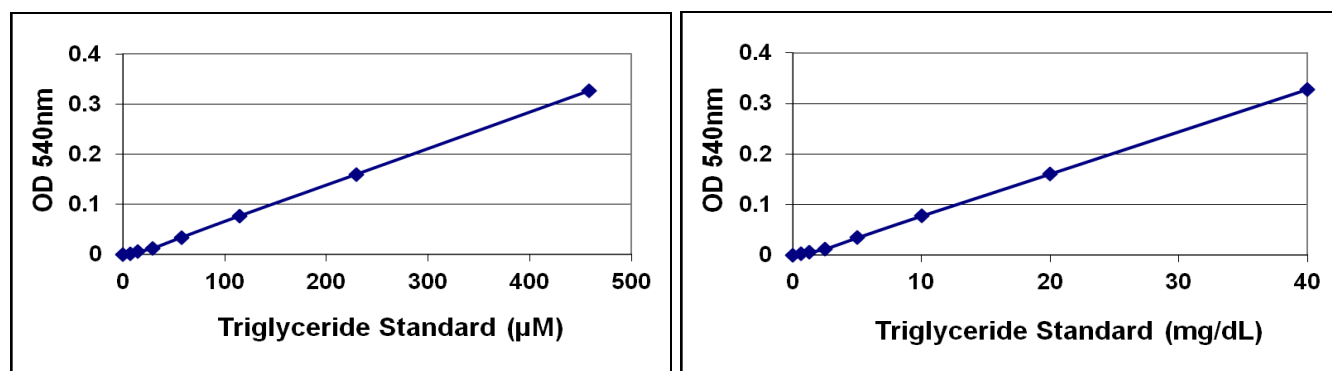


Figure 1: Triglyceride Standard Curve. Triglyceride standard curve was performed according to the Assay Protocol, data plotted in μM (left) and mg/dL (right).

References

- Bucolo, G. and David, H. (1973) *Clin. Chem.* **19(5)**, 476-482.
- Fossati, P. and Prencipe, L. (1982) *Clin. Chem.* **28(10)**, 2077-2080.
- McGowan, M.W. et al. (1983) *Clin. Chem.* **29(3)**, 538-542.
- Mendez, A.J., Cabeza, C., and Hsia, S.L. (1986) *Anal. Biochem.* **156**, 386-389.

Recent Product Citations

- Ruathong, W. et al. (2024). The probiotic *Lactobacillus rhamnosus* SD11 alleviates the progression of liver and colon damage through modulation of inflammation and tight junction proteins in streptozotocin-induced diabetic mice. *PLoS One*. **19(11)**:e0313395. doi: 10.1371/journal.pone.0313395.
- Song, L. et al. (2024). KLF1 Activates RAC3 to Mediate Fatty Acid Synthesis and Enhance Cisplatin Resistance in Bladder Cancer Cells. *Am J Mens Health*. **18(5)**:15579883241273305. doi: 10.1177/15579883241273305.
- Giraldo, N.D. et al. (2024). Functional assessment of a whey protein hydrolysate: In vivo responses in lipid regulation, oxidant defense, and neural protection in *Caenorhabditis elegans*. *Int Dairy J*. doi: 10.1016/j.idairyj.2024.105921.

4. Shelton, C.D. et al. (2023). An early-life microbiota metabolite protects against obesity by regulating intestinal lipid metabolism. *Cell Host Microbe*. **31**(10):1604-1619.e10. doi: 10.1016/j.chom.2023.09.002.
5. Fitzgerald, H. et al. (2023). Resolvin D2-GPR18 Enhances Bone Marrow Function and Limits Steatosis and Hepatic Collagen Accumulation in Aging. *Am J Pathol*. doi: 10.1016/j.ajpath.2023.08.011.
6. Nopparat, J. et al. (2023). Probiotics of *Lactobacillus paracasei* SD1 and *Lactobacillus rhamnosus* SD11 attenuate inflammation and β -cell death in streptozotocin-induced type 1 diabetic mice. *PLoS One*. **18**(4):e0284303. doi: 10.1371/journal.pone.0284303.
7. Fahrner, A. et al. (2023). microRNA-501 controls myogenin+/CD74+ myogenic progenitor cells during muscle regeneration. *Mol Metab*. **71**:101704. doi: 10.1016/j.molmet.2023.101704.
8. Fuke, N. et al. (2023). Inter-Day Variation in the Fasting Plasma Lipopolysaccharide Concentration in the Morning Is Associated with Inter-Day Variation in Appetite in Japanese Males: A Short-Term Cohort Study. *Metabolites*. **13**(3):395. doi: 10.3390/metabo13030395.
9. Lin, B. et al. (2023) Vitamin E Supplement Protects Against Gestational Diabetes Mellitus in Mice Through nuclear factor-erythroid factor 2-related factor 2/heme oxygenase-1 Signaling Pathway. *Diabetes Metab Syndr Obes*. **16**:565-574. doi: 10.2147/DMSO.S397255.
10. Lee, D. et al. (2023). Pemafibrate prevents choroidal neovascularization in a mouse model of neovascular age-related macular degeneration. *PeerJ*. doi: 10.7717/peerj.14611.
11. Lee, D. et al. (2022). Retinal degeneration induced in a mouse model of ischemia-reperfusion injury and its management by pemafibrate treatment. *FASEB J*. **36**(9):e22497. doi: 10.1096/fj.202200455RRR.
12. Elsyade, R. et al. (2021). Hazards of Chronic Exposure to Nonylphenol: Concomitant Effect on Non-alcoholic Fatty Liver Disease in Male Albino Rats. *Open Access Maced J Med Sci*. **9**(A):548-555. doi: 10.3889/oamjms.2021.6237.
13. Kimura, M. et al. (2021). Feeding Rats Medium-Chain Triglycerides and Tomato Powder Increases Liver Lycopene Content and Reduces the Expression of Genes Related to Lipid Metabolism in the Liver. *ACS Food Sci. Technol*. doi: 10.1021/acsfoodscitech.0c00155.
14. Lee, H.B. et al. (2020). Molokhia leaf extract prevents gut inflammation and obesity. *J Ethnopharmacol*. doi: 10.1016/j.jep.2020.112866.
15. Li, J. et al. (2020). Salsalate reverses metabolic disorders in a mouse model of non-alcoholic fatty liver disease through AMPK activation and caspase-6 activity inhibition. *Basic Clin Pharmacol Toxicol*. doi: 10.1111/bcpt.13535.
16. Kim, D.Y. et al. (2020). Angiotensin AT1 receptor antagonism by losartan stimulates adipocyte browning via induction of apelin. *J Biol Chem*. doi: 10.1074/jbc.RA120.013834.
17. Greco, C.M. et al. (2020). A non-pharmacological therapeutic approach in the gut triggers distal metabolic rewiring capable of ameliorating diet-induced dysfunctions encompassed by metabolic syndrome. *Sci Rep*. **10**(1):12915. doi: 10.1038/s41598-020-69469-y.
18. Choi, E.M. et al. (2020). Orientin reduces the inhibitory effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on adipogenic differentiation and insulin signaling pathway in murine 3T3-L1 adipocytes. *Chem Biol Interact*. doi: 10.1016/j.cbi.2020.108978.
19. Sohag, M. et al. (2019). Potential Antidiabetic Activities of Probiotic Strains, *L. acidophilus* and *L. bulgaricus* against Fructose-Fed Hyperglycemic Rats. *Food Nutr Sci*. **10**:1419-1432. doi: 10.4236/fns.2019.1012101.
20. Al-Maiahy, T.J. et al. (2019). Prolactin and risk of preeclampsia: A single institution, cross-sectional study. *Asian Pac J Reprod* 2019. **8**:112-7. doi: 10.4103/2305-0500.259168.

21. Kim, H. et al. (2019). Persistent changes in liver methylation and microbiome composition following reversal of diet-induced non-alcoholic-fatty liver disease. *Cell Mol Life Sci.* doi: 10.1007/s00018-019-03114-4.
22. Nopparat, J. et al. (2019). Ethanolic extracts of *Pluchea indica* (L.) leaf pretreatment attenuates cytokine-induced β -cell apoptosis in multiple low-dose streptozotocin-induced diabetic mice. *PLoS One.* **14**(2):e0212133. doi: 10.1371/journal.pone.0212133.
23. Singh, A. et al. (2019). Host genetics and diet composition interact to modulate gut microbiota and predisposition to metabolic syndrome in spontaneously hypertensive stroke-prone rats. *FASEB J.* fj201801627RRR. doi: 10.1096/fj.201801627RRR.
24. Sugihara, M. et al. (2019). The AAA+ ATPase/ubiquitin ligase mysterin stabilizes cytoplasmic lipid droplets. *J Cell Biol.* **218**(3):949-960. doi: 10.1083/jcb.201712120.
25. Pant, A. et al. (2019). Farnesol induces fatty acid oxidation and decreases triglyceride accumulation in steatotic HepaRG cells. *Toxicol Appl Pharmacol.* **365**:61-70. doi: 10.1016/j.taap.2019.01.003.
26. Choi, E.M. et al. (2018). Glabridin attenuates antiadipogenic activity induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in murine 3T3-L1 adipocytes. *J Appl Toxicol.* **38**(11):1426-1436. doi: 10.1002/jat.3664.
27. Singh, A. et al. (2018). Inulin fiber dose-dependently modulates energy balance, glucose tolerance, gut microbiota, hormones and diet preference in high-fat-fed male rats. *J Nutr Biochem.* **59**:142-152. doi: 10.1016/j.jnutbio.2018.05.017.
28. Lee, E-S. et al (2018). Amelioration of obesity in high-fat diet-fed mice by chestnut starch modified by amylosucrase from *Deinococcus geothermalis*. *Food Hydrocolloids.* **75**: 22-32.
29. Ilavenil, S. et al. (2017). Ferulic acid in *Lolium multiflorum* inhibits adipogenesis in 3T3-L1 cells and reduced high-fat-diet-induced obesity in Swiss albino mice via regulating p38MAPK and p44/42 signal pathways. *Journal of Functional Foods.* **37**: 293-302.
30. Gulhane, M. et al. (2016). High fat diets induce colonic epithelial cell stress and inflammation that is reversed by IL-22. *Sci Rep.* doi:10.1038/srep28990.

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