NOTE: Revisions to
"Preparation of Standard"

Product Manual

Lipid Quantification Kit (Colorimetric)

Catalog Number

STA-613 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipids are a diverse group of molecules that include monoglycerides, diglycerides, trigylcerides, fats, sterols, and others. Not only do lipids define and preserve cellular membrane integrity, but they are also involved in cellular processes such as membrane trafficking, signal transduction, apoptosis, and energy storage. Perturbation in the metabolism of lipids has been linked to many diseases such as cancer, diabetes, Alzheimer's disease, and coronary heart disease.

In order to study lipids, they must often be extracted first from tissues or cellular cultures (1) and then quantified. Some methods for lipid quantification have been described that are quite sensitive, however they require expensive equipment such as an HPLC machine, light scattering detection technology, or a latroscan TLC-FID analyser (2,3).

Cell Biolabs' Lipid Quantification Kit measures the lipid content (unsaturated fatty acids only) of samples using the sulfo-phospho-vanillin method (4), resulting in a simple colorimetric readout amenable to multi-well plate detection. First, a crude or purified lipid source is applied to a 96 well plate. Then concentrated sulfuric acid is added and the samples are heated to solubilize and prime the total lipid sample, followed by the addition of vanillin in an acid solution. The lipids react with vanillin in the presence of the acids to form a colorimetric product that is easily detected on a microplate reader. Each kit provides sufficient reagents to perform 100 assays including standards and unknown samples.

Related Products

1. STA-612: Lipid Extraction Kit (Chloroform Free)
2. STA-384: Total Cholesterol Assay Kit (Colorimetric)
3. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
4. STA-398: Free Glycerol Assay Kit (Colorimetric)
5. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

Kit Components

1. Vanillin Reagent (Part No. 261301): One 10 mL amber bottle.
2. Purified Lipid Standard (Part No. 261302): One 100 µL vial at 100 g/dL.

Materials Not Supplied

1. Concentrated Sulfuric Acid (18M)
2. DMSO or other organic solvent
3. Glass tubes, 15 mL conical tubes, microcentrifuge tubes, or 96 well plates
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 1000 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir

Storage

Store the kit at -20ºC.

Preparation of Samples

- Plasma: Collect blood with an anticoagulant such as citrate, EDTA, heparin or oxalate 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.
- 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.
- Cultured Cells or Tissue Samples: Prepare lipids using Cell Biolabs' Lipid Extraction Kit (Cat. #STA-612) or Folch method (Ref. 1).

Preparation of Purified Lipid Standard

Thaw the Purified Lipid Standard at room temperature. First, dilute the Purified Lipid Standard 1:10 in dimethyl sulfoxide (DMSO) or desired solvent for a 10 g/dL Purified Lipid Standard. (e.g., add 5 µL of the 100 g/dL Purified Lipid Standard to 45 µL of DMSO or other solvent). Use the 10 g/dL Purified Lipid Standard to prepare a series of the remaining lipid standards in the concentration range of 0 to 300 mg/dL in dimethyl sulfoxide (DMSO) or desired solvent according to Table 1 below.

Standard Tubes	10 g/dL Purified Lipid Standard (µL)	DMSO or other organic solvent (µL)	Standard (mg/dL)
1	12	388	300
2	200 of Tube #1	200	150
3	200 of Tube #2	200	75
4	200 of Tube #3	200	37.5
5	200 of Tube #4	200	18.75
6	200 of Tube #5	200	9.38
7	200 of Tube #6	200	4.69
8	0	100	0

Table 1. Preparation of Purified Lipid Standard Curve.

Assay Protocol

Note: Sulfuric acid is highly corrosive and can damage certain types of plastics. Avoid using plastics that are sensitive to sulfuric acid, and test plastics prior to attempting this assay by adding 100 µL of sulfuric acid and heating to 90ºC for 10 minutes. Sulfuric acid should be handled with care. Gloves, a lab coat, and protective eyewear should be worn during handling. Sulfuric acid should be stored in glassware only and be pipetted in a fume hood.

1. Add 15 µL of samples or standards into microcentrifuge tubes or a 96-well plate.

   Note: For samples in DMSO, skip steps 2 and 3 and proceed to step 4.

2. Incubate samples and standards uncovered at 90°C for 30 minutes to completely evaporate organic solvents.

   Note: This step is optional for aqueous, non-organic based samples, which will not evaporate during heating.

3. Transfer samples to 4°C for 5 minutes.
4. Add 150 µL of 18M sulfuric acid.
5. Incubate samples at 90°C for 10 minutes.
6. Transfer samples to 4°C for 5 minutes.
7. Transfer 100 µL of each standard and unknown sample into a clean 96-well plate.
8. Read samples at OD 540 nm to determine background.
9. Add 100 µL of Vanillin Reagent and mix carefully.

   Note: The Vanillin Reagent tends to precipitate and may require incubation at 37°C for 15-30 minutes to go into solution.

10. Incubate samples at 37°C for 15 minutes.
11. Read samples at OD 540 nm to determine signal.
12. Subtract background from signal.

Example of Results

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

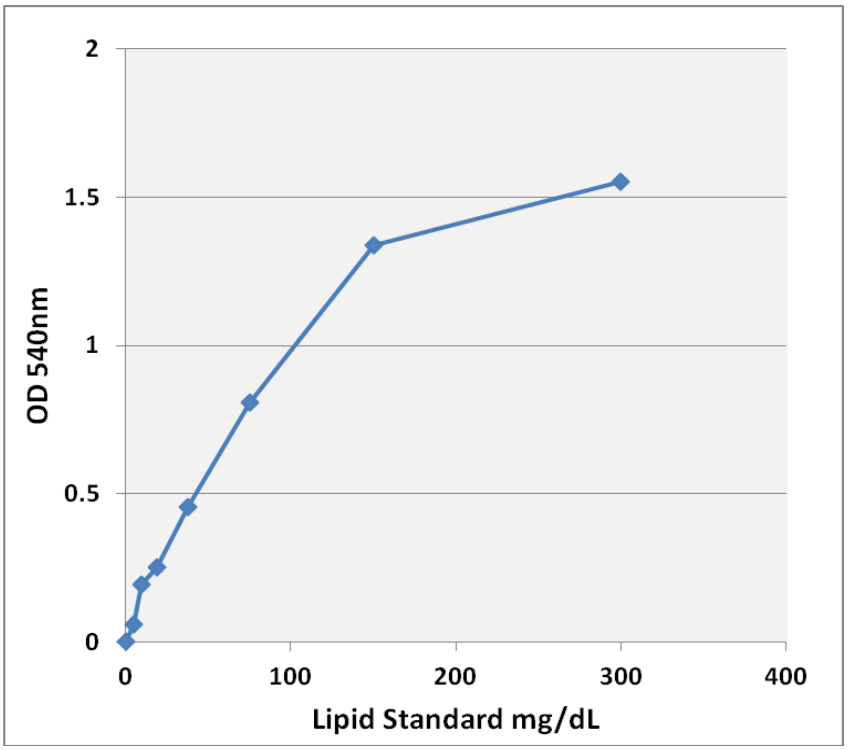

Figure 1: Lipid Quantification Kit Standard Curve.

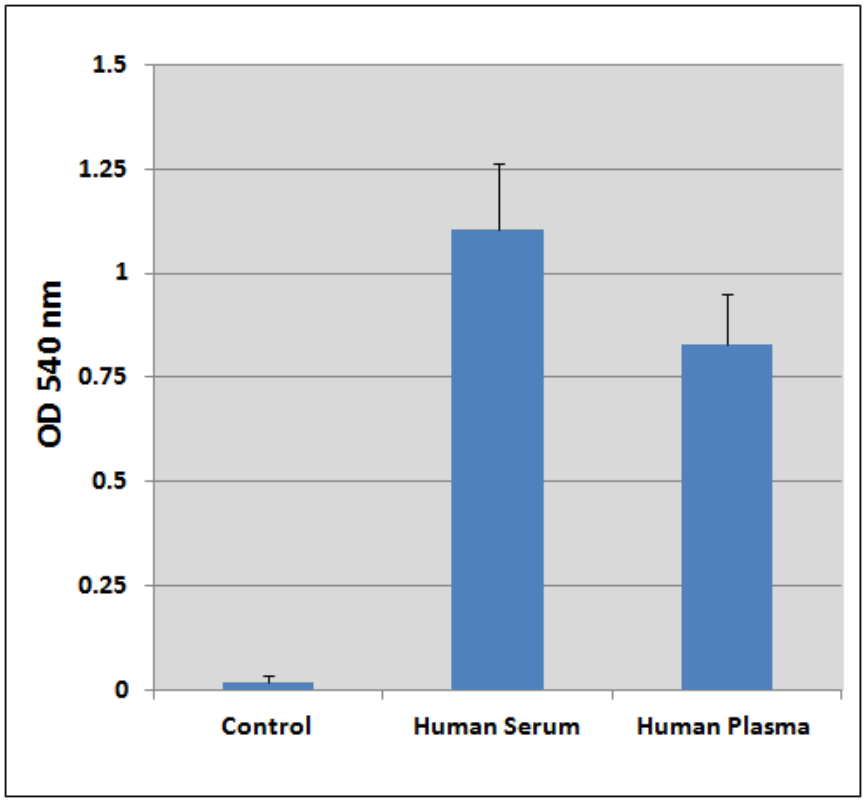

Figure 2: Detection of lipids from Human Serum or Human Plasma. Fifteen microliters of undiluted Human Serum, Human Plasma, or Negative Control buffer were analyzed using the Lipid Quantification Kit (Colorimetric).

References

1. Folch J, Lees M, and Slone Stanley GH (1956) *J. Biol. Chem*. **226**, 497-509.
2. Lutzke BS and Brauler JM. (1990) *J. Lipid Res.*, **31**, 212-2130
3. Fraser AJ, Tocher DR, and Sargent JR (1985) *J. Exp. Marine Biol. And Ecol*. **88**, 91-99.
4. Chabrol E and Charonnat R (1937) Presse Med. **45**, 1713-1714.

Recent Product Citations

1. Gao, H. et al. (2023). Outer membrane vesicles from a mosquito commensal mediate targeted killing of Plasmodium parasites via the phosphatidylcholine scavenging pathway. *Nat Commun*. **14**(1):5157. doi: 10.1038/s41467-023-40887-6.
2. Soetikno V. et al. (2023). Alpha-Mangosteen lessens high-fat/high-glucose diet and low-dose streptozotocin induced-hepatic manifestations in the insulin resistance rat model. *Pharm Biol*. **61**(1):241-248. doi: 10.1080/13880209.2023.2166086.
3. Barlin, M. et al. (2022). Proteins in Tumor-Derived Plasma Extracellular Vesicles Indicate Tumor Origin. *Mol Cell Proteomics*. **22**(1):100476. doi: 10.1016/j.mcpro.2022.100476.
4. Mansouri, S. et al. (2022). MPYS Modulates Fatty Acid Metabolism and Immune Tolerance at Homeostasis Independent of Type I IFNs. *J Immunol*. doi: 10.4049/jimmunol.2200158.

5. Mohamed, M.F. et al. (2022). Presence of CrkI-containing microvesicles in squamous cell carcinomas could have ramifications on tumor biology and cancer therapeutics. *Sci Rep*. **12**(1):4803. doi: 10.1038/s41598-022-08905-7.
6. Barrett, E. et al. (2021). Uteroplacental Insufficiency with Hypoxia Upregulates Placental PPARγ-KMT5A Axis in the Rat. *Reprod Sci*. doi: 10.1007/s43032-020-00434-w.
7. Kumar, S. et al. (2021). Cocaine-Specific Effects on Exosome Biogenesis in Microglial Cells. *Neurochem Res*. doi: 10.1007/s11064-021-03231-2.
8. Pishavar, E. et al. (2020). Comparison Study of Stem Cell-Derived Extracellular Vesicles for Enhanced Osteogenic Differentiation. *Tissue Eng Part A*. doi: 10.1089/ten.TEA.2020.0194.
9. Kawabe, Y. et al. (2020). (Bombyx Insulin-Like Peptide) Increases the Respiration Rate Through Facilitation of Carbohydrate Catabolism in Bombyx mori. *Front Endocrinol (Lausanne)*. **10**:150. doi: 10.3389/fendo.2019.00150.
10. Inagaki, T. et al. (2020). Gut microbial pulse provides nutrition for parental provisioning in incipient termite colonies. *Behav Ecol Sociobiol*. **74**(6). doi: 10.1007/s00265-020-02843-y.
11. Zhu, Q. et al. (2020). Bupivacaine inhibits angiogenesis through oxidative stress-dependent inhibition of Akt/mTOR and activation of AMPK. *Fundam Clin Pharmacol*. doi: 10.1111/fcp.12554.
12. Nair, P. et al. (2020). Metabolic responses to long-term food deprivation in subterranean and surface amphipods. *Subterr Biol*. **33**:1–15. doi: 10.3897/subtbiol.33.48483.
13. Langridge, T.D. et al. (2019). Toward understanding polymer micelle stability: Density ultracentrifugation offers insight into polymer micelle stability in human fluids. *J Control Release*. **319**:157-167. doi: 10.1016/j.jconrel.2019.12.038.
14. Ahmadpour, A. et al. (2019). Study of the Circulatory Energy Balance Indicators and Hepatic Fat Content in Dromedary Camel during Late Pregnancy and Early Lactation. *Small Ruminant Research*. doi: 10.1016/j.smallrumres.2019.08.018.
15. Huang, Z. et al. (2019). ALOX12 inhibition sensitizes breast cancer to chemotherapy via AMPK activation and inhibition of lipid synthesis. *Biochem Biophys Res Commun*. pii: S0006-291X (19)30743-0. doi: 10.1016/j.bbrc.2019.04.101.
16. Fanning, S. et al. (2019). Lipidomic Analysis of α-Synuclein Neurotoxicity Identifies Stearoyl CoA Desaturase as a Target for Parkinson Treatment. *Mol Cell*. **73**(5):1001-1014.e8. doi: 10.1016/j.molcel.2018.11.028.
17. Stranska, R. et al. (2018). Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human plasma. *J Transl Med*. **16**(1):1. doi: 10.1186/s12967-017-1374-6.
18. Yang, X. et al. (2018). Inhibiting 6-phosphogluconate dehydrogenase selectively targets breast cancer through AMPK activation. *Clin Transl Oncol*. **20**(9):1145-1152. doi: 10.1007/s12094-018-1833-4.
19. Tosic, M. et al. (2018). Lsd1 regulates skeletal muscle regeneration and directs the fate of satellite cells. *Nat Commun*. **9**(1):366. doi: 10.1038/s41467-017-02740-5.
20. Gupta, K.H. et al. (2017). Apoptosis and compensatory proliferation signaling are coupled by CrkI-containing microvesicles. *Dev. Cell* **41**(6):674-684.
21. Duteil, D. et al. (2016). Lsd1 ablation triggers metabolic reprogramming of brown adipose tissue. *Cell Reports* **17**:1008-1021.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com