Product Manual

Lipid Quantification Kit (Fluorometric)

Catalog Number

STA-617 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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 analyzer (2,3).

Cell Biolabs' Lipid Quantification Kit (Fluorometric) specifically measures the neutral lipid content (see Figure 2) of samples using a lipid binding molecule that fluoresces brightly only when bound to lipids, resulting in a simple fluorescence readout. First, a crude or purified lipid source is applied to a 96-well plate. Then organic solvents are removed, followed by the addition of the fluorescent reagent. Lipid binding to the fluorescent reagent results in an increase in fluorescence that is easily detected on a microplate reader. This kit has a detection sensitivity of 3.9 mg/dL and is at least 10 times more sensitive than the Vanillin method (4). 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-613: Lipid Quantification Kit (Colorimetric)
3. STA-241: Human Low Density Lipoprotein
4. STA-242: Human Very Low Density Lipoprotein
5. STA-243: Human High Density Lipoprotein
6. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
7. STA-390: Total Cholesterol Assay Kit (Fluorometric)
8. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
9. STA-394: HDL Cholesterol Assay Kit
10. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
11. STA-399: Free Glycerol Assay Kit (Fluorometric)
12. STA-619: Free Fatty Acid Assay Kit (Fluorometric)
13. STA-600: Phosphatidylcholine Assay Kit

Kit Components

1. Fluorometric Reagent (100X) (Part No. 261701): One 200 µL vial
2. Lipid Standard (Part No. 261702): One 100 µL vial at 20 g/dL neutral lipids

Materials Not Supplied

1. Methanol
2. Chloroform
3. Isopropanol
4. Glass tubes, 15 mL conical tubes, microcentrifuge tubes, or 96 well plates
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 1000 µL adjustable multichannel micropipette with disposable tips
7. Multichannel micropipette reservoir
8. 96-well black or fluorescence microtiter plate
9. A heat block or oven capable of heating to 55°C.
10. Fluorescent microplate reader capable of reading 490 nm (excitation) and 585 nm (emission)

Storage

Store the kit at 4ºC.

Preparation of Reagents

- 1X Fluorometric Reagent: Thaw the Fluorometric Reagent (100X) stock at 37ºC for 5 minutes. Dilute 100 fold with water.
- Methanol/Chloroform Mixture (only for testing organic phase samples): Mix 2 volumes of methanol with 1 volume of chloroform in a glass bottle. Store at room temperature.

  Note: Methanol/Chloroform Mixture is toxic. Avoid skin contact or inhalation and use a fume hood for preparation.

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. Dilute as necessary in water.
- 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. Dilute as necessary in water.
- Cultured cells or tissue samples: Prepare lipids using Cell Biolabs' Lipid Extraction Kit (Cat. #STA-612) or Folch method (Ref. 1). Dilute as necessary in Methanol/Chloroform Mixture.

Preparation of Lipid Standards

Prepare a dilution series of Lipid Standards in the concentration range of 0 to 500 mg/dL (Table 1).

- If testing aqueous phase samples (plasma, serum or purified lipoproteins), prepare standards in distilled water.
- If testing organic phase samples (lipid extracts or purified lipids), prepare standards in Methanol/Chloroform Mixture (see Preparation of Reagents).

Standard Tubes	20 g/dL Lipid Standard (µL)	Water or Methanol/Chloroform (µL)	Standard (mg/dL)
1	10	390	500
2	200 of Tube #1	200	250
3	200 of Tube #2	200	125
4	200 of Tube #3	200	62.5
5	200 of Tube #4	200	31.3
6	200 of Tube #5	200	15.6
7	200 of Tube #6	200	7.8
8	200 of Tube #7	200	3.9
9	0	200	0

Table 1. Preparation of Lipid Standard Curve.

Assay Protocol

I. For aqueous phase samples such as plasma, serum, or purified lipoproteins

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknown and standard, should be assayed in duplicate.
2. Add 40 µL of standards or unknown samples to a 96-well plate.
3. Add 200 µL of 1X Fluorometric Reagent and pipet up and down 5 times to mix.
4. Incubate at room temperature for 5-15 minutes protected from light.
5. Read the plate at 490 nm excitation and 585 nm emission with a fluorescent plate reader.
6. Calculate the concentration of lipids within samples by comparing the sample fluorescence to the standard curve.

II. For organic phase samples such as lipid extracts or purified lipids

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknown and standard, should be assayed in duplicate.
2. Add 40 µL of standards or unknown samples to a 96-well plate.
3. Incubate the plate at 55°C for 20-30 minutes to fully evaporate the organic solvent.
4. Incubate the plate at 4°C for 2-3 minutes.
5. Add 40 µL of isopropanol. Mix the well by pipetting up and down 10 times.
6. Add 200 µL of 1X Fluorometric Reagent and pipet up and down 5 times to mix.

7. Incubate at room temperature for 5-15 minutes protected from light.
8. Read the plate at 490 nm excitation and 585 nm emission with a fluorescent plate reader.
9. Calculate the concentration of lipids within samples by comparing the sample fluorescence to the standard curve.

Example of Results

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

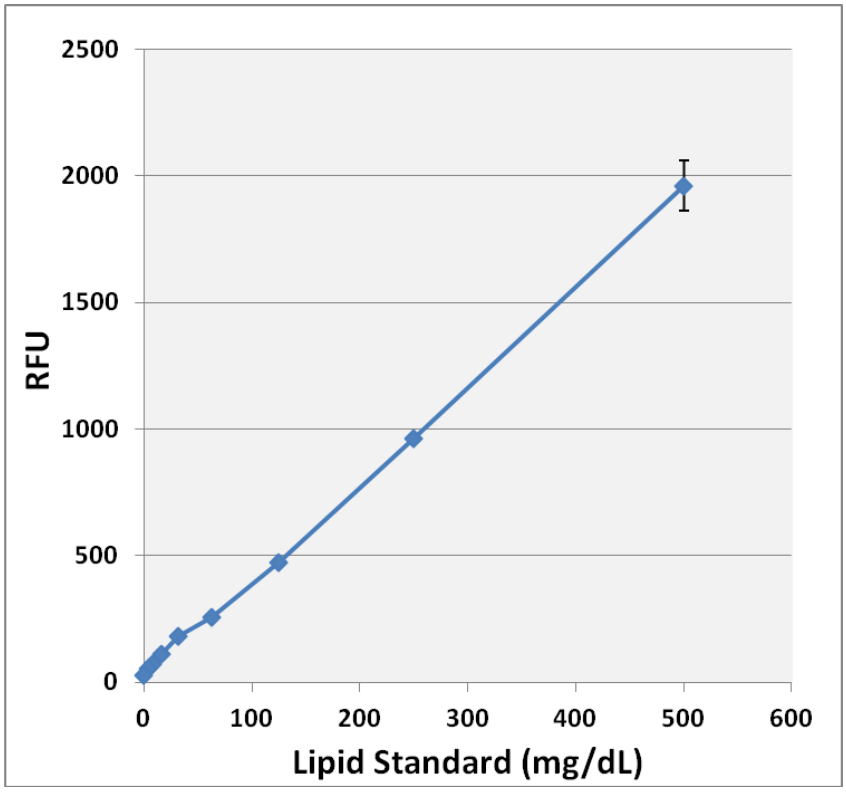

Figure 1: Lipid Quantification Kit (Fluorometric) standard curve in methanol/chloroform.

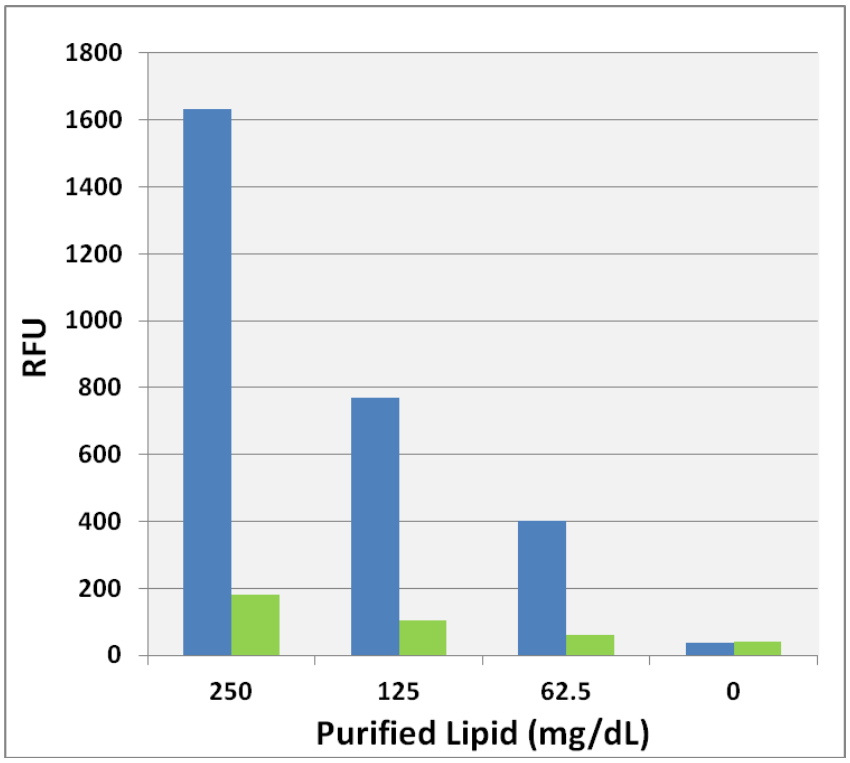

Figure 2: Detection of triolein or lysophosphatidylcholine. Purified triolein (blue bars) or lysophosphatidylcholine (green bars) were diluted in Methanol/Chloroform and analyzed using the Lipid Quantification Kit (Fluorometric).

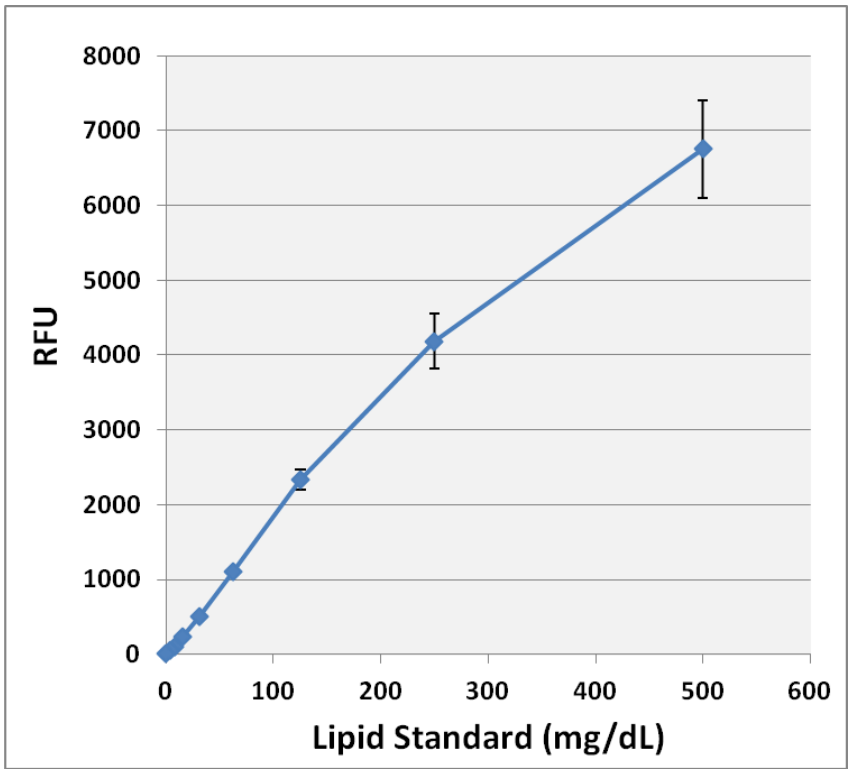

Figure 3: Lipid Quantification Kit (Fluorometric) standard curve in water.

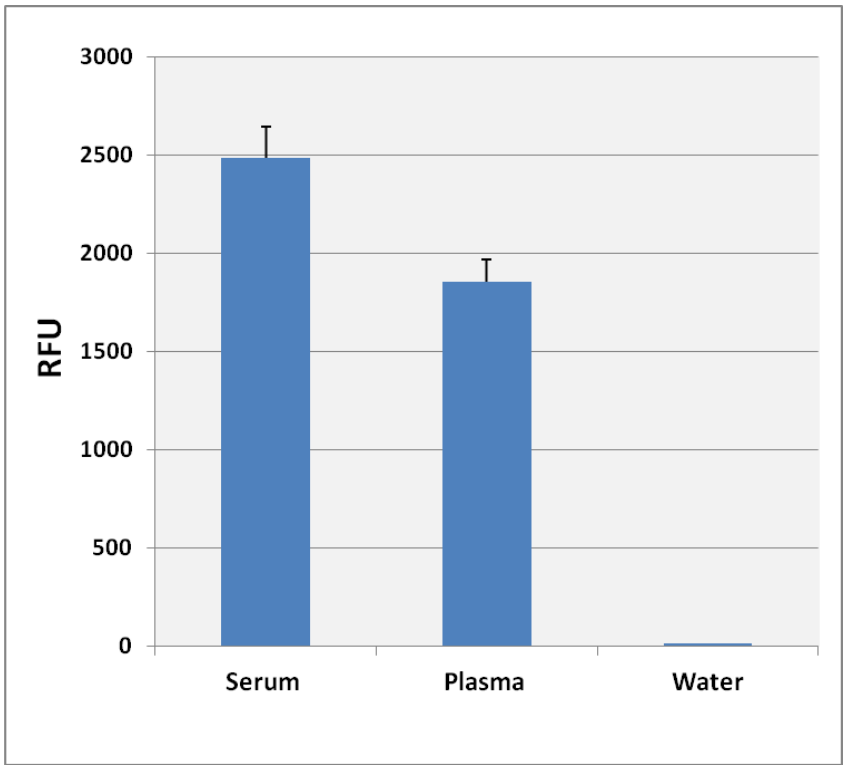

Figure 4: Detection of lipids in aqueous samples. Undiluted human serum, human plasma, and water were analyzed using the Lipid Quantification Kit (Fluorometric).

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 citation

Ali, D. A. et al. (2016). Structural and functional abnormalities of hepatic tissues of male Wistar rats fed on hyper whey and super amino anabolic protein. *Nutrition.* doi:10.1016/j.nut.2016.01.017.

Warranty

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