

**NOTE: Revisions to
“Preparation of Standard”**

Product Manual

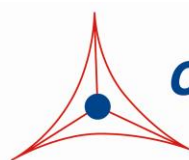
Lipid Quantification Kit (Colorimetric)

Catalog Number

STA-613

100 assays

**FOR RESEARCH USE ONLY
Not for use in diagnostic procedures**



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Creating Solutions for Life Science Research

Introduction

Lipids are a diverse group of molecules that include monoglycerides, diglycerides, triglycerides, 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 μ L 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 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
5. 50 μ L to 1000 μ L adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir

Storage

Store the kit at -20°C.

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 90°C 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.

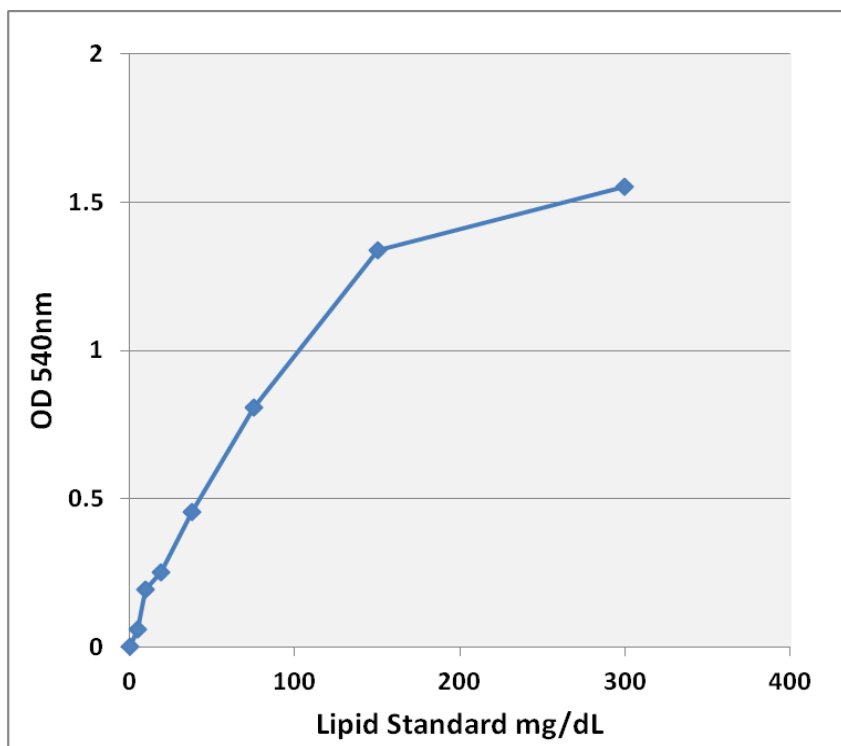


Figure 1: Lipid Quantification Kit Standard Curve.

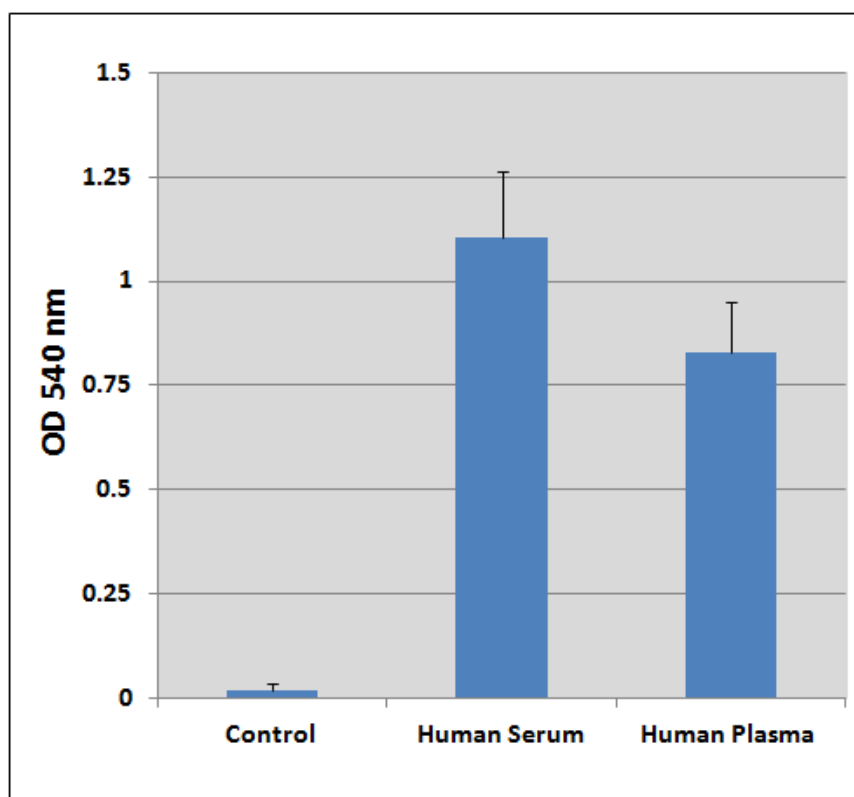


Figure 2: Detection of lipids from Human Serum or Human Plasma. Fifteen microliters of undiluted Human Serum, Human Plasma, or Negative Control buffer were analyzed using the Lipid Quantification Kit (Colorimetric).

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Recent Product Citations

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