Product Manual

Lipid Extraction & Polar/Neutral Lipid Separation Combo Kit (Chloroform Free)

Catalog Number

MET-5009-C **50 preps**

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 cultured cells. Traditionally, organic extraction by the Folch method (Ref. 1) has been preferred, but this method has several disadvantages. First, it extracts lipids to a bottom organic phase, forcing penetration of the upper protein-containing phase during purification and causing contamination of lipid samples. As a result, low purity lipid samples can hamper downstream lipid analysis by clogging instruments such as high pressure liquid chromatographs (HPLCs). In addition, the Folch method uses chloroform as the organic phase solvent. Long-term exposure to chloroform by inhalation has resulted in effects on the liver such as hepatitis and jaundice. Furthermore, chloroform has been demonstrated to be carcinogenic in animals, causing in an increase in kidney and liver tumors. In fact, the United States Environmental Protection Agency (EPA) has classified chloroform as a Group B2, probable human carcinogen.

Cell Biolabs' Lipid Extraction & Polar/Neutral Lipid Separation Combo Kit combines total organic lipid extraction with further separation of polar and neutral lipids. The lipid extraction procedure circumvents the above disadvantages by first extracting total lipids to an upper organic phase (making it amenable to high throughput extraction) that is chloroform free. A crude lipid source such as serum or tissue culture cell pellet is resuspended in a proprietary alcohol. After adding a proprietary organic compound, the mixture is centrifuged to gravitationally separate the phases. The recovered upper organic phase (containing total lipids) is dried, and then polar lipids are separated from neutral lipids by adding both a proprietary secondary alcohol and a proprietary secondary organic compound. The top neutral lipid layer is removed from the bottom polar lipid layer and both are dried and resuspended for downstream analysis.

Each kit provides sufficient reagents to isolate up to 50 preps based on a 100 µL sample size. Larger sample sizes may be used (see Table 1) yielding proportionally fewer preps per kit.

Related Products

1. MET-5009: Polar/Neutral Lipid Separation Kit
2. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
3. STA-384: Total Cholesterol Assay Kit (Colorimetric)
4. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
5. STA-394: HDL Cholesterol Assay Kit
6. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
7. STA-398: Free Glycerol Assay Kit (Colorimetric)
8. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
9. STA-600: Phosphatidylcholine Assay Kit

10. STA-612: Lipid Extraction Kit
11. STA-613: Lipid Quantification Kit (Colorimetric)

Kit Components

1. Lipid Extraction Reagent A (Part No. 261201): One 25 mL amber glass bottle.
2. Lipid Extraction Reagent B (Part No. 261202): One 75 mL amber glass bottle.
3. Lipid Extraction Reagent C (Part No. 261203): One 25 mL bottle.
4. Neutral Lipid Extraction Reagent (Part No. 50091A): One 55 mL amber glass bottle.
5. Polar Lipid Extraction Reagent (20X) (Part No. 50092A): One 0.75 mL vial.

Materials Not Supplied

1. Glass tubes, 15 mL conical tubes, or microcentrifuge tubes
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 1000 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Phosphate buffered saline (PBS)
6. Tube vortexer
7. Organic solvent (such as chloroform, butanol, or cyclohexane)
8. 100% Methanol

Storage

Store the entire kit at room temperature. To avoid possible leakage store bottles upright.

Preparation of Samples

- 1X Polar Lipid Extraction Reagent: Dilute the Polar Lipid Extraction Reagent (20X) by transferring 0.75 mL to a 50 mL conical tube containing 14.25 mL of 100% Methanol. Mix to homogeneity. Store the 1X Polar Lipid Extraction Reagent at room temperature for up to six months.

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. Samples should be extracted immediately or may be stored at -80°C.
- 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 extracted immediately or may be stored at -80°C.

- Cultured Cells: Pellet 5-10 x 10^7 cells at 1000 x g for 5 minutes. Wash cells once with 1X PBS, and resuspend final cell pellet with 100 µL 1X PBS. Perform the extraction as described in the kit protocol below.
- Tissues: Carefully mince the tissue into small fragments with a scalpel/razor blade and weigh in a 50 mL conical tube. Add PBS to a final tissue concentration of 2 mg/mL. Homogenize the tissue at 4°C. Perform the extraction from the whole tissue homogenate as described in the kit protocol below.

Protocol

I. Lipid Extraction

The protocol below is written for a 100 µL sample size. Refer to Table 1 below for the appropriate dispensing volumes when working with other sample sizes.
Note: Number of preps per kit will be reduced proportionally with increasing sample volumes.

Sample Volume:	100 µL	500 µL	1 mL
Step 2: Lipid Extraction Reagent A	500 µL	2.5 mL	5 mL
Step 3: Lipid Extraction Reagent B	250 µL	1.25 mL	2.5 mL
Step 4: Lipid Extraction Reagent B	250 µL	1.25 mL	2.5 mL
Step 5: Lipid Extraction Reagent C	500 µL	2.5 mL	5 mL
Step 8: Lipid Extraction Reagent B	530 µL	2.65 mL	5.3 mL
Step 11: Lipid Extraction Reagent B	420 µL	2.1 mL	4.2 mL

Table 1. Dispensing Volumes for Various Sample Sizes.

1. Add 100 µL of serum, plasma, cell suspension, or whole tissue homogenate to a tube.
2. Add 500 µL of Lipid Extraction Reagent A and vortex for 10 minutes (a tube shaker or vortexer is recommended).
3. Add 250 µL of Lipid Extraction Reagent B and vortex for 5 minutes.
4. Add an additional 250 µL of Lipid Extraction Reagent B and vortex for 5 minutes.
5. Add 500 µL of Lipid Extraction Reagent C and vortex for 5 minutes.
6. Centrifuge the tube at 1000 x g for 5 minutes.
7. Carefully remove the top organic layer containing lipid to a new tube.
8. Add 530 µL of Lipid Extraction Reagent B to the remaining (bottom) aqueous layer and vortex for 5 minutes.
9. Centrifuge the tube at 1000 x g for 5 minutes.

10. Carefully remove the top organic layer containing lipid and pool with the first organic layer from step 7.
11. Add 420 µL of Lipid Extraction Reagent B to the remaining (bottom) aqueous layer and vortex for 5 minutes.
12. Centrifuge the tube at 1000 x g for 5 minutes.
13. Carefully remove the top organic layer containing lipid and pool with the first two organic layers.
14. Leave the pooled organic layer tube open and dry in a vacuum concentrator or in a dry 37ºC incubator overnight (or until dry).

II. Polar/Neutral Lipid Separation

The protocol below is written for lipids extracted from a 100 µL sample size (i.e. starting volume of plasma, serum, cell lysate or tissue homogenate in section I above). Refer to Table 2 below for the appropriate dispensing volumes when working with other sample sizes.
Note: Number of preps per kit will be reduced proportionally with increasing sample volumes.

Sample Volume:	100 µL	500 µL	1 mL
Step 1: Neutral Lipid Extraction Reagent	150 µL	0.75 mL	1.5 mL
Step 3: 1X Polar Lipid Extraction Reagent	300 µL	1.5 mL	3.0 mL
Step 4: Neutral Lipid Extraction Reagent	300 µL (repeated 3 times)	1.5 mL (repeated 3 times)	3.0 mL (repeated 3 times)

Table 2. Dispensing Volumes for Various Input Sample Sizes.

1. Add 150 µL of Neutral Lipid Extraction Reagent to the dried pooled total lipids from step 14 of the Total Lipid Extraction Protocol above.
2. Vortex the sample gently for 10 minutes.
3. Add 300 µL of 1X Polar Extraction Reagent.
4. Add 300 µL of Neutral Lipid Extraction Reagent.
5. Vortex lightly for 10 seconds.
6. Incubate for 30 seconds and visually ensure that phase separation is complete.
7. Pipette off the top layer (containing neutral lipids) and transfer to a new labeled tube.
8. Repeat steps 4 through 7 two additional times. Pool the top layers.
9. Dry the pooled upper neutral lipid fraction and remaining lower polar lipid fraction in a vacuum concentrator or in a dry 37ºC incubator overnight (or until dry).
10. Resuspend the dried fractions in an organic solvent such as butanol or cyclohexane.

Note: Chloroform may be used for resuspension if desired.

Example of Results

The following figures demonstrate typical results of various assays using samples prepared with the Lipid Extraction and Polar/Neutral Lipid Separation Combo Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

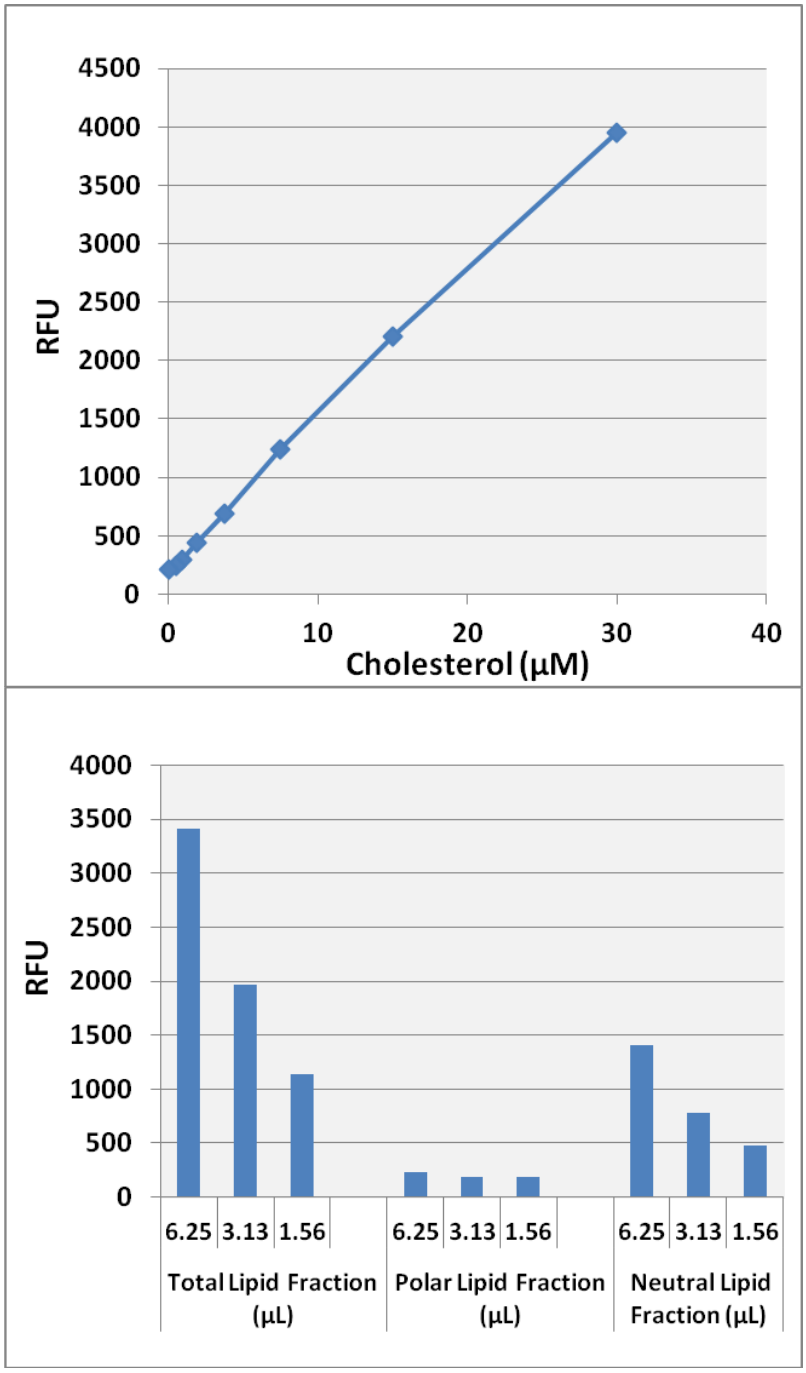

Figure 1: Total Cholesterol Assay (Cat. #STA-390) Performed on Extracted Lipids. Top: Cholesterol Standard Curve. **Bottom**: Total, polar and neutral lipids were extracted from homogenized chicken liver using the Lipid Extraction Kit (Chloroform Free) (Cat. #STA-612) and tested for the presence of Cholesterol according to the Assay Protocol.

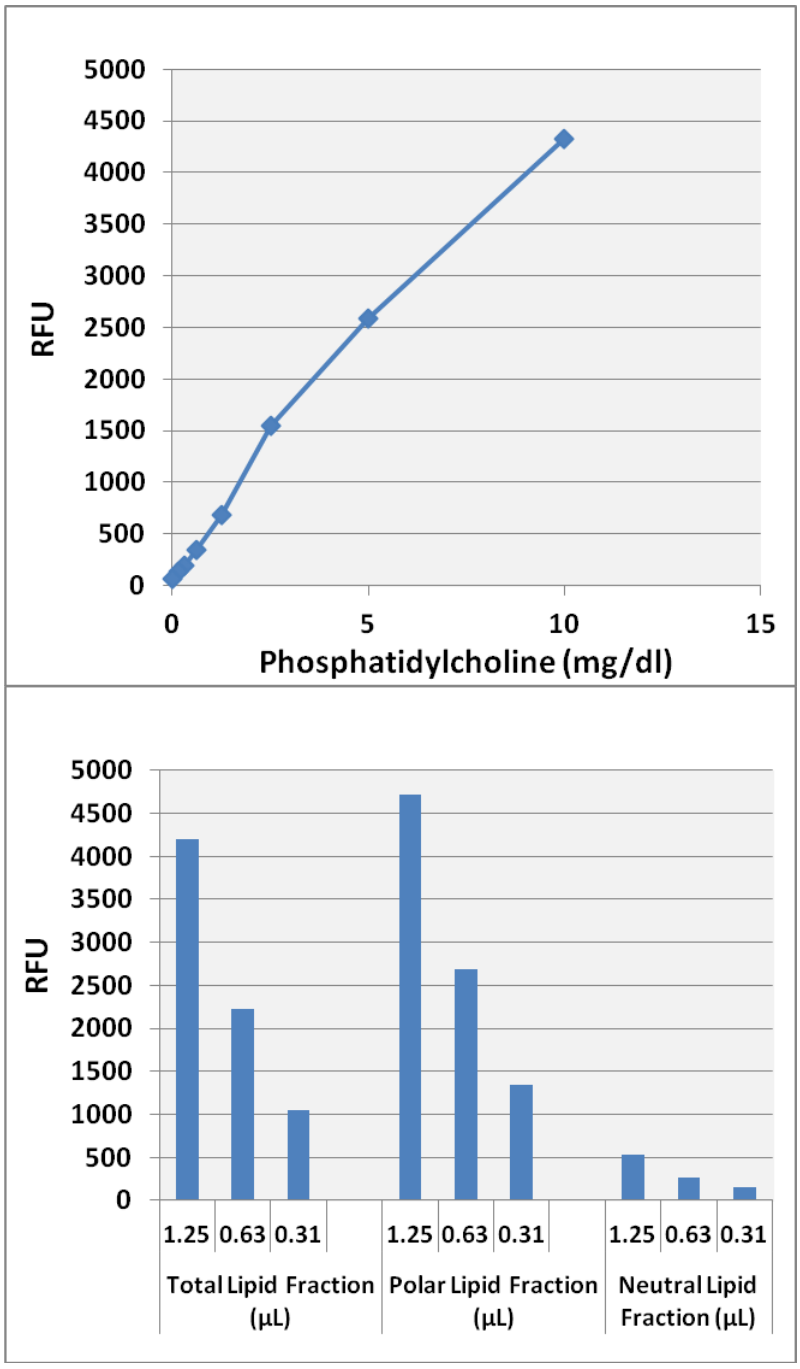

Figure 2: Phosphatidylcholine Assay (Cat. #STA-600) Performed on Extracted Lipids. Top: Phosphatidylcholine (PC) Standard Curve. **Bottom**: Total, polar and neutral lipids were extracted from homogenized chicken liver using the Lipid Extraction Kit (Chloroform Free) (Cat. #STA-612) and tested for the presence of Phosphatidylcholine according to the Assay Protocol.

References

1. Folch J, Lees M, and Slone Stanley GH (1956) *J. Biol. Chem*. **226**, 497-509.
2. Iverson SJ, Lang SLC, and Cooper MH (2001) *J. Lipid Res*. **36**, 1283-1287.
3. Bang DY, Byeon SK, Moon MH (2014) *J. Chromatogr A*. **28**, 1331
4. Reis A, Rudnitskaya A, Blackburn GJ, Mohd Fauzi N, Pitt AR, Spickett CM (2013) *J. Lipid Res.*, **54**, 1812-1824.

Warranty

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