Product Manual

Polar/Neutral Lipid Separation Kit

Catalog Number

MET-5009 **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. Organic extraction may be performed using the Folch method (Ref. 1), or by using our Lipid Extraction Kit (Chloroform Free, Cat. #STA-612).

Cell Biolabs' Polar/Neutral Lipid Separation Kit separates polar and neutral lipids starting from total organic lipid pools previously extracted by one of the aforementioned methods. The 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-C: Lipid Extraction & Polar/Neutral Lipid Separation Combo Kit
2. STA-241: Human Low Density Lipoprotein
3. STA-243: Human High Density Lipoprotein
4. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
5. STA-384: Total Cholesterol Assay Kit (Colorimetric)
6. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
7. STA-394: HDL Cholesterol Assay Kit
8. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
9. STA-398: Free Glycerol Assay Kit (Colorimetric)
10. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
11. STA-600: Phosphatidylcholine Assay Kit
12. STA-612: Lipid Extraction Kit
13. STA-613: Lipid Quantification Kit (Colorimetric)

Kit Components

1. Neutral Lipid Extraction Reagent (Part No. 50091A): One 55 mL amber glass bottle.
2. 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. Tube vortexer
6. Organic solvent (such as chloroform, butanol, or cyclohexane)
7. 100% Methanol

Storage

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

Preparation of Reagents

- 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

- Total Organic Lipids: Extract organic lipids using desired method, e.g. Folch method or Cell Biolabs' Lipid Extraction Kit (Cat. #STA-612). Leave the pooled organic layer tube open and dry in a vacuum concentrator or in a dry 37ºC incubator overnight (or until dry).

Protocol

The protocol below is written based on lipids extracted from a starting sample size of 100 µL (i.e. serum, plasma, cell suspension, or whole tissue homogenate). 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 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 1. Dispensing Volumes for Various Input Sample Sizes.

1. Add 150 µL of Neutral Lipid Extraction Reagent to the dried pooled total lipid sample.
2. Vortex the sample gently for 10 minutes.
3. Add 300 µL of 1X Polar Lipid 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 Polar/Neutral Lipid Separation Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

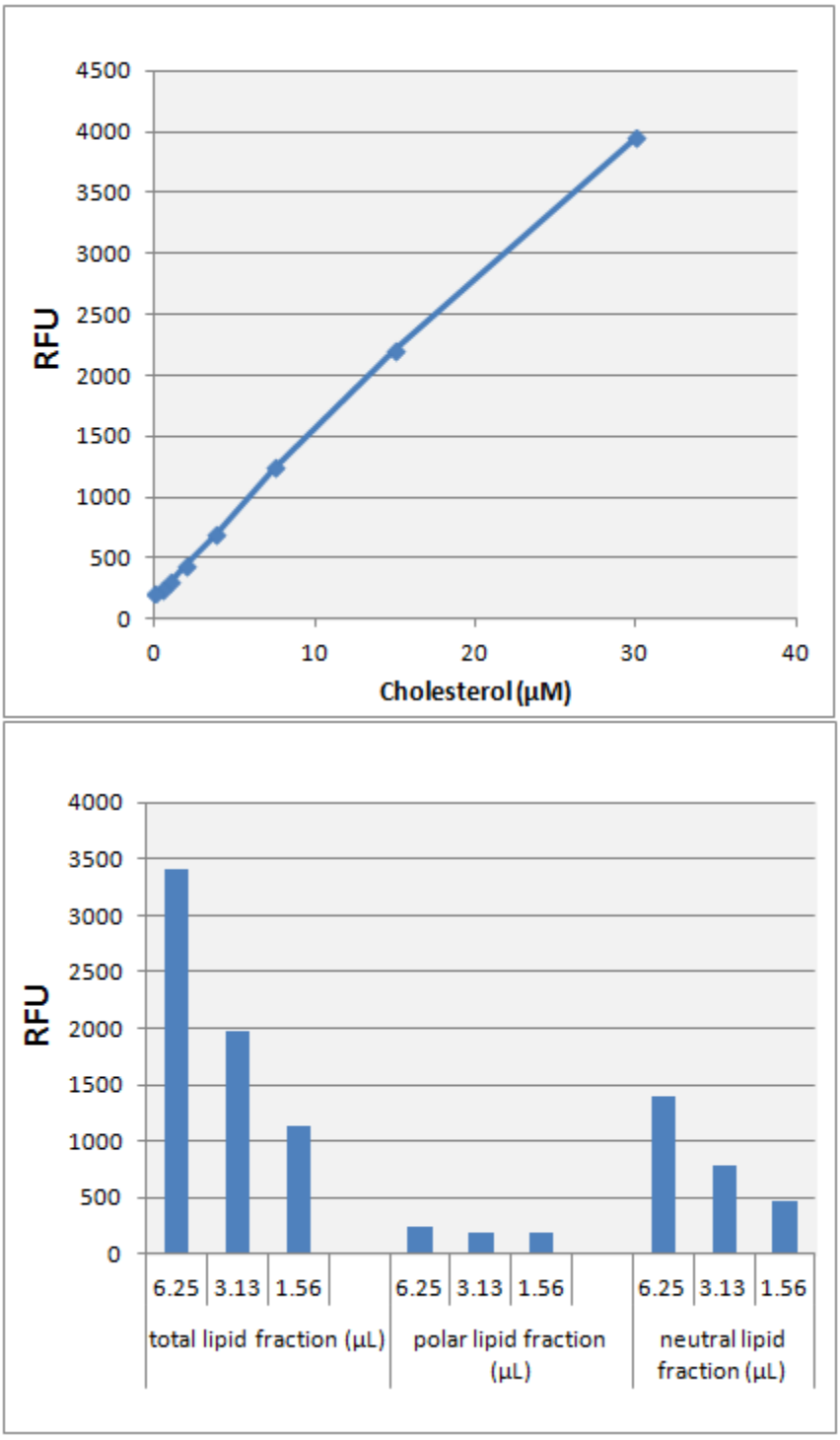

Figure 1: Total Cholesterol Assay (Cat. #STA-390) Performed on Extracted Lipids. Top: Cholesterol Standard Curve. **Bottom**: Total, polar and neutral lipids were extracted from homogenized chicken liver using the Lipid Extraction Kit (Chloroform Free) (Cat. #STA-612) and tested for the presence of Cholesterol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

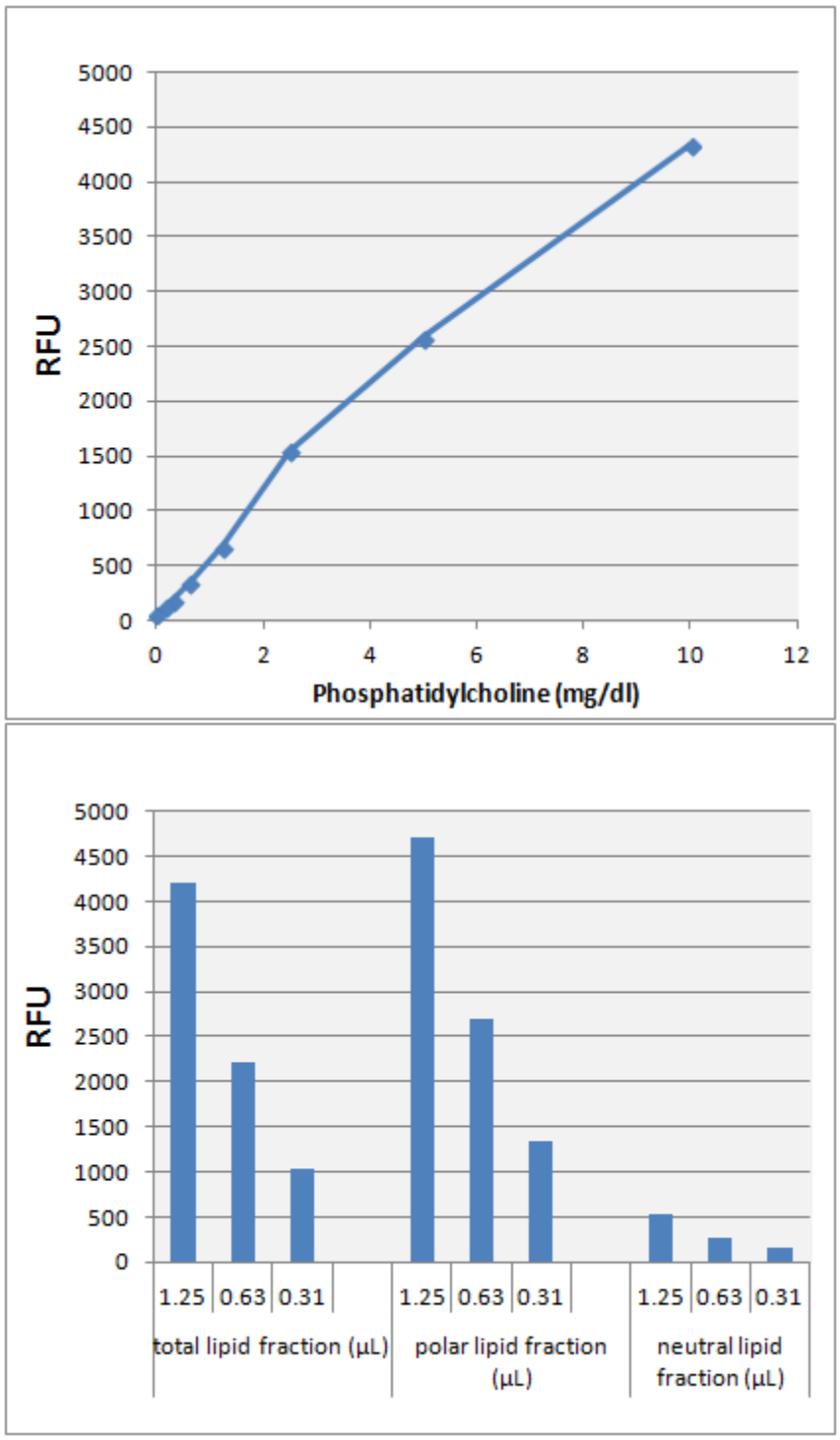

Figure 2: Phosphatidylcholine Assay (Cat. #STA-600) Performed on Extracted Lipids. Top: Phosphatidylcholine (PC) Standard Curve. **Bottom**: Total, polar and neutral lipids were extracted from homogenized chicken liver using the Lipid Extraction Kit (Chloroform Free) (Cat. #STA-612) and tested for the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