

**NOTE: Revision to  
Kit Components**

---

**Product Manual**

# **Lipid Extraction Kit (Chloroform Free)**

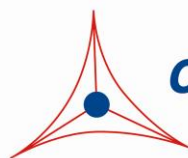
**Catalog Number**

**STA-612**

**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, 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 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 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 Kit isolates total lipids by organic extraction, but circumvents the above disadvantages by extracting 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 is then dried and resuspended for downstream lipid analysis. Each kit provides sufficient reagents to isolate up to 50 preps based on a 100  $\mu$ L sample size. Larger sample sizes may be used (see Table 1) yielding proportionally fewer preps per kit.

## **Related Products**

1. STA-613: Lipid Quantification Kit (Colorimetric)
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

## **Kit Components**

1. Lipid Extraction Reagent A (Part No. 261201): One 25 mL amber glass bottle.
2. Lipid Extraction Reagent B1 (Part No. 261204): Two 30 mL amber glass bottles.
3. Lipid Extraction Reagent B2 (Part No. 261205): One 20 mL amber glass bottle.
4. Lipid Extraction Reagent C (Part No. 261203): One 25 mL bottle.

## **Materials Not Supplied**

1. Glass tubes, 15 mL conical tubes, or microcentrifuge tubes
2. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
3. 50  $\mu$ L to 1000  $\mu$ L 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)

## **Storage**

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

## **Preparation of Reagents**

- Lipid Extraction Reagent B: Combine 3 parts of Lipid Extraction Reagent B1 with 1 part of Lipid Extraction Reagent B2 in an amber glass bottle. Mix well. Keep reagents protected from light until ready for use.

## **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<sup>6</sup> cells at 1000 x g for 5 minutes. Wash cells once with 1X PBS, and resuspend final cell pellet with 100  $\mu$ L 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.

## **Extraction Protocol**

The protocol below is written for a 100  $\mu\text{L}$  sample size. Refer to Table 1 below for the appropriate dispensing volumes when working with other sample sizes.

*Note: The number of preps per kit will be reduced proportionally with increasing sample volumes.*

<b>Sample Volume:</b>	<b>100 <math>\mu\text{L}</math></b>	<b>500 <math>\mu\text{L}</math></b>	<b>1 mL</b>
Step 2: Lipid Extraction Reagent A	500 $\mu\text{L}$	2.5 mL	5 mL
Step 3: Lipid Extraction Reagent B	250 $\mu\text{L}$	1.25 mL	2.5 mL
Step 4: Lipid Extraction Reagent B	250 $\mu\text{L}$	1.25 mL	2.5 mL
Step 5: Lipid Extraction Reagent C	500 $\mu\text{L}$	2.5 mL	5 mL
Step 8: Lipid Extraction Reagent B	530 $\mu\text{L}$	2.65 mL	5.3 mL
Step 11: Lipid Extraction Reagent B	420 $\mu\text{L}$	2.1 mL	4.2 mL

**Table 1. Dispensing Volumes for Various Sample Sizes.**

1. Add 100  $\mu\text{L}$  of serum, plasma, cell suspension, or whole tissue homogenate to a tube.
2. Add 500  $\mu\text{L}$  of Lipid Extraction Reagent A and vortex for 10 minutes (a tube shaker or vortexer is recommended).
3. Add 250  $\mu\text{L}$  of Lipid Extraction Reagent B (see Preparation of Reagents section) and vortex for 5 minutes.
4. Add an additional 250  $\mu\text{L}$  of Lipid Extraction Reagent B and vortex for 5 minutes.
5. Add 500  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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 37°C incubator overnight (or until dry).

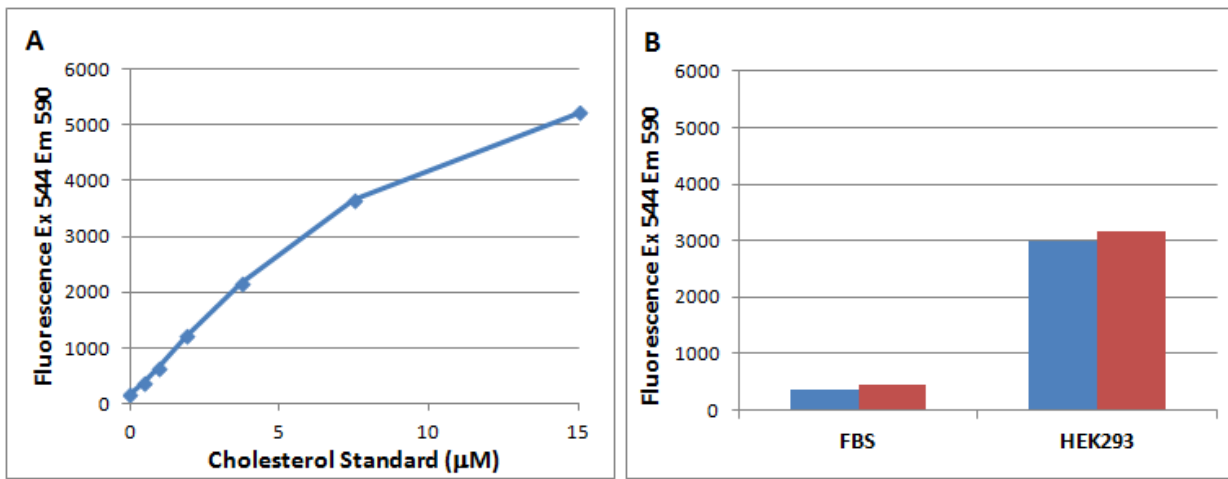
*Note: Recovery volume should be approximately 1600  $\mu$ L. For faster dry down, split samples into two equal parts.*

15. Resuspend the lipid extract in an organic solvent such as butanol or cyclohexane.

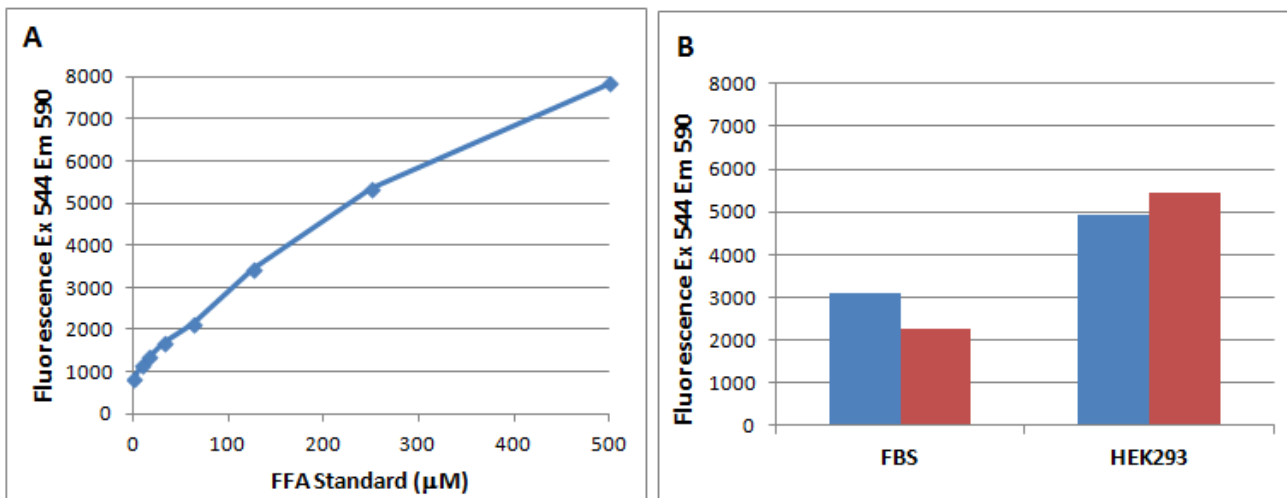
*Note: Chloroform may be used for resuspension if desired.*

### **Example of Results**

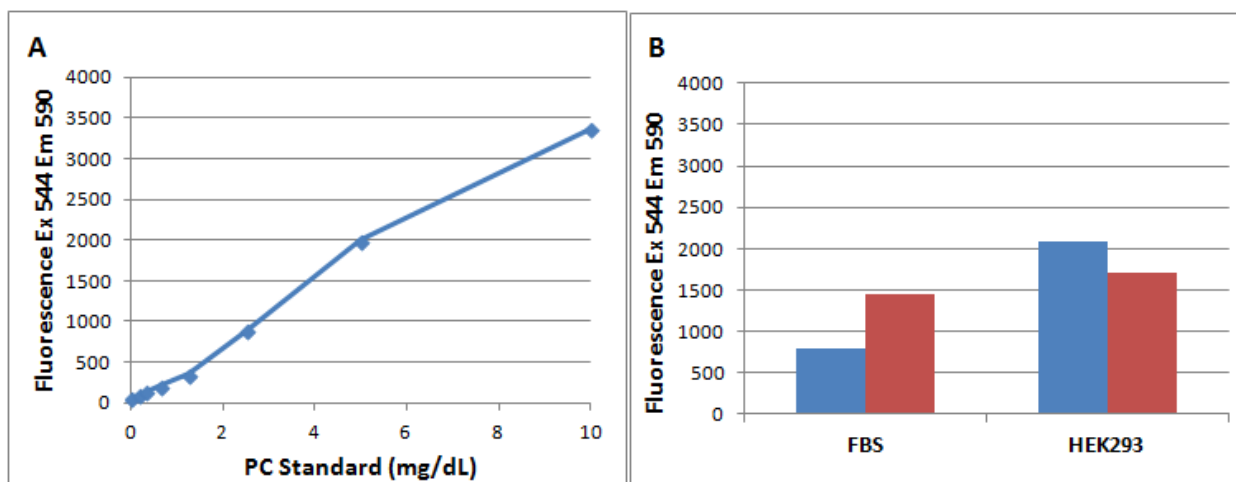
The following figures demonstrate typical results of various assays using samples prepared with the Lipid Extraction Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: Total Cholesterol Assay (Cat. #STA-390) Performed on Extracted Lipids.** (A) Cholesterol Standard Curve. (B) Lipids extracted from Fetal Bovine Serum (FBS) or HEK293 Cells prepared by the Folch Method (Blue Bars) or the Lipid Extraction Kit, Chloroform-Free (Red Bars) were tested for the presence of Cholesterol according to the Assay Protocol.



**Figure 2: Free Fatty Acid Assay (Cat. #STA-619) Performed on Extracted Lipids.** (A) Free Fatty Acid (FFA) Standard Curve. (B) Lipids extracted from FBS or HEK293 Cells prepared by the Folch Method (Blue Bars) or the Lipid Extraction Kit, Chloroform-Free (Red Bars) were tested for the presence of FFA according to the Assay Protocol.



**Figure 3: Phosphatidylcholine Assay (Cat. #STA-600) Performed on Extracted Lipids.** (A) Phosphatidylcholine (PC) Standard Curve. (B) Lipids extracted from FBS or HEK293 Cells prepared by the Folch Method (Blue Bars) or the Lipid Extraction Kit, Chloroform-Free (Red Bars) were 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.

## Recent Product Citations

1. Han, L. et al. (2023). Lipid droplet-associated lncRNA LIPTER preserves cardiac lipid metabolism. *Nat Cell Biol.* doi: 10.1038/s41556-023-01162-4.
2. Trombetti, S. et al. (2023). Over-Expressed GATA-1S, the Short Isoform of the Hematopoietic Transcriptional Factor GATA-1, Inhibits Ferroptosis in K562 Myeloid Leukemia Cells by Preventing Lipid Peroxidation. *Antioxidants (Basel)*. **12**(3):537. doi: 10.3390/antiox12030537.
3. 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.
4. Dozio, E. et al. (2022). A Wide-Proteome Analysis to Identify Molecular Pathways Involved in Kidney Response to High-Fat Diet in Mice. *Int J Mol Sci.* **23**(7):3809. doi: 10.3390/ijms23073809.
5. Seki, M. et al. (2022). Local free fatty acids trigger the expression of lipopolysaccharide-binding protein in murine white adipose tissue. *Biosci Microbiota Food Health.* doi: 10.12938/bmfh.2021-061.

6. Fukami, H. et al. (2021). Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia. *Cell Rep Med.* **2**(11):100446. doi: 10.1016/j.xcrm.2021.100446.
7. Bajaj, A. et al. (2020). Method of extraction and proteome profiling of mycobacteria using liquid chromatography-high resolution mass spectrometry. *SN Appl. Sci.* doi: 10.1007/s42452-020-03691-1.
8. Jun, Y. et al. (2020). Leukocyte-Mediated Combined Targeted Chemo and Gene Therapy for Esophageal Cancer. *ACS Appl Mater Interfaces.* doi: 10.1021/acsami.0c15419.
9. Greco, C.M. et al. (2020). A non-pharmacological therapeutic approach in the gut triggers distal metabolic rewiring capable of ameliorating diet-induced dysfunctions encompassed by metabolic syndrome. *Sci Rep.* **10**(1):12915. doi: 10.1038/s41598-020-69469-y.
10. 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.
11. Srisomboon, Y. et al. (2020). Fungal allergen-induced IL-33 secretion involves cholesterol-dependent, VDAC-1-mediated ATP release from the airway epithelium. *J Physiol.* doi: 10.1113/JP279379.
12. Srisowanna, N. et al. (2019). The Effect of Estrogen on Hepatic Fat Accumulation during Early Phase of Liver Regeneration after Partial Hepatectomy in Rats. *Acta Histochem. Cytochem.* **52**(4):67-75. doi:10.1267/ahc.19018.
13. 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.
14. 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.
15. Matoba, K. et al. (2017). Adipose KLF15 Controls Lipid Handling to Adapt to Nutrient Availability. *Cell Rep.* **21**(11):3129-3140. doi: 10.1016/j.celrep.2017.11.032.
16. Tyszka-Czochara, M. et al. (2017). Metformin and caffeic acid regulate metabolic reprogramming in human cervical carcinoma SiHa/HTB-35 cells and augment anticancer activity of Cisplatin via cell cycle regulation. *Food Chem. Toxicol.* **106**:260-272.
17. Maki, T. et al. (2017). Renoprotective effect of a novel selective PPAR $\alpha$  modulator K-877 in db/db mice: A role of diacylglycerol-protein kinase C-NAD(P)H oxidase pathway. *Metabolism Clinical and Experimental.* **71**: 33–45.
18. Tyszka-Czochara, M. et al. (2017). Caffeic acid expands anti-tumor effect of Metformin in human metastatic cervical carcinoma HTB-34 cells: Implications of AMPK activation and impairment of fatty acids de novo biosynthesis. *Int J Mol Sci.* **18**(2) doi: 10.3390/ijms.
19. Pamir, N. et al. (2015). Granulocyte macrophage-colony stimulating factor-dependent dendritic cells restrain lean adipose tissue expansion. *J. Biol. Chem.* doi:10.1074/jbc.M115.645820.

## **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](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2014-2023: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.