

**NOTE: Revision to  
Kit Components**

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**Product Manual**

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

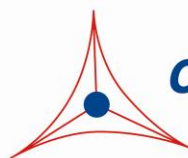
**Catalog Number**

**STA-612**

**50 preps**

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

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**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-384: Total Cholesterol Assay Kit (Colorimetric)
3. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
4. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
5. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

## **Kit Components (shipped at room temperature)**

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. Phosphate buffered saline (PBS)
3. Tube vortexer
4. 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 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.

## **Extraction Protocol**

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

<b>Sample Volume:</b>	<b>100 µL</b>	<b>500 µL</b>	<b>1 mL</b>
Step 2: Lipid Extraction Reagent A	500 µL	2.5 mL	5 mL

Step 3: Lipid Extraction Reagent B	250 $\mu$ L	1.25 mL	2.5 mL
Step 4: Lipid Extraction Reagent B	250 $\mu$ L	1.25 mL	2.5 mL
Step 5: Lipid Extraction Reagent C	500 $\mu$ L	2.5 mL	5 mL
Step 8: Lipid Extraction Reagent B	530 $\mu$ L	2.65 mL	5.3 mL
Step 11: Lipid Extraction Reagent B	420 $\mu$ L	2.1 mL	4.2 mL

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

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

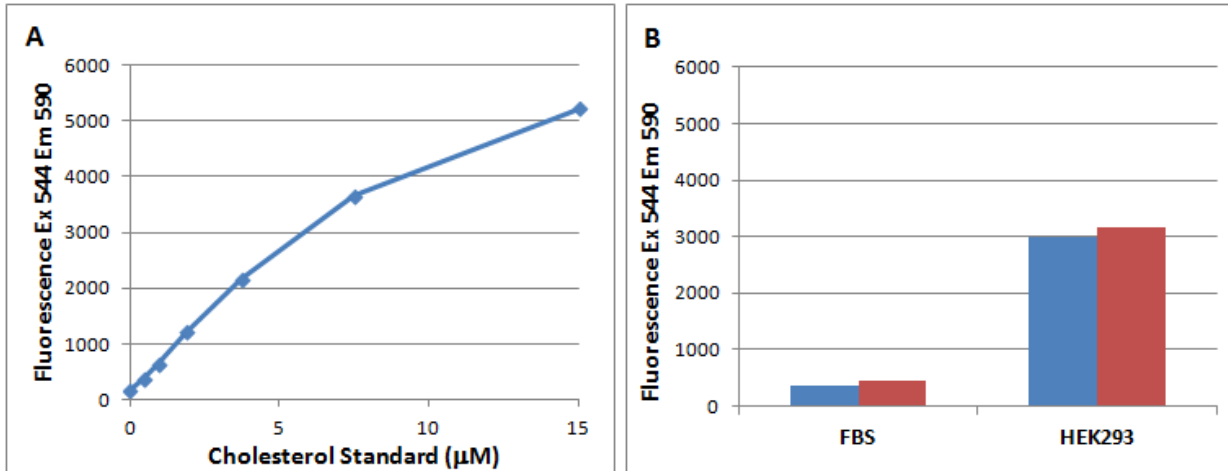
*Note: Recovery volume should be approximately 1600  $\mu$ L. For faster dry down, split samples into two equal parts.*

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

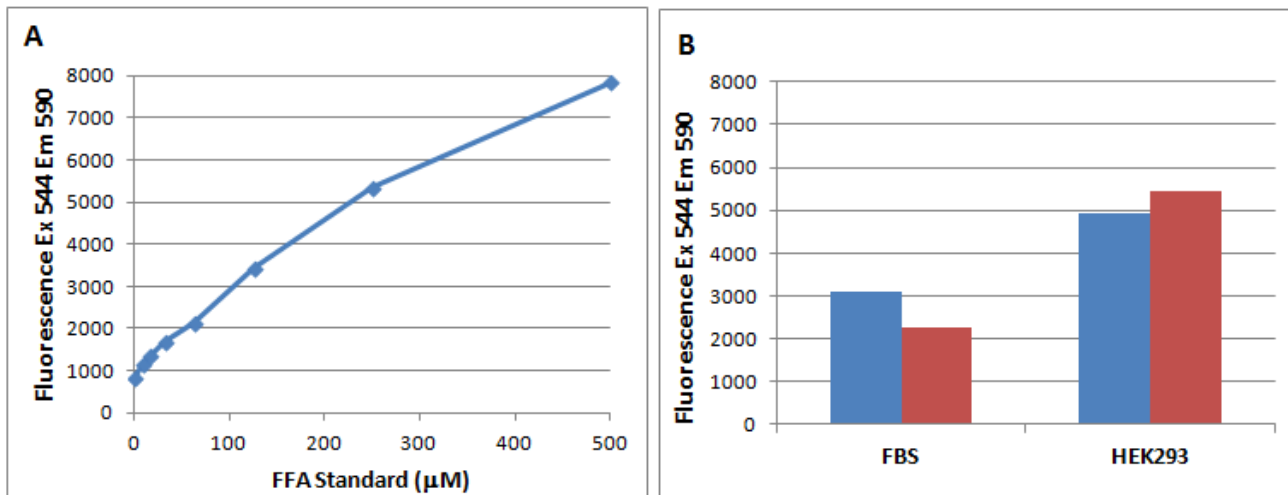
*Note: Chloroform may be used for resuspension if desired.*

## Example of Results

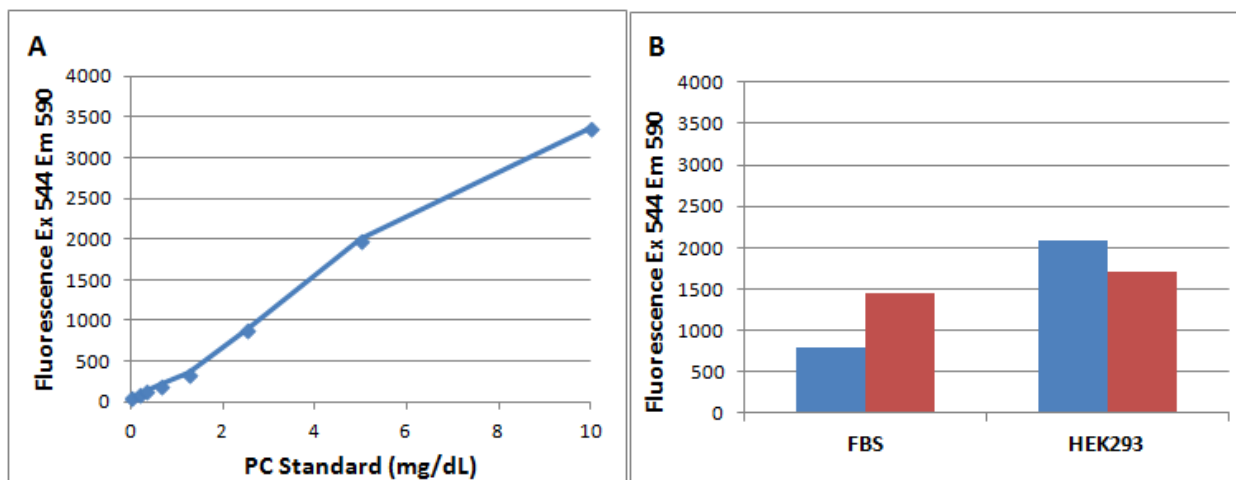
The following figures demonstrate typical results of various assays using samples prepared with the Lipid Extraction Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



**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.

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