Lipid Extraction Kit (Chloroform Free)

(Catalog # BN00493; 50 assays; Store at RT)

I. Introduction:

Lipids are a group of naturally occurring molecules that include monoglycerides, diglycerides, trigylcerides, fats, sterols and others. Lipids define and preserve cellular membrane integrity and play an important role in cellular processes such as membrane trafficking, signal transduction and energy storage. Dysregulation of lipid metabolism is linked to many diseases like cancer, diabetes and heart disease. Traditionally, the Folch method of organic extraction of lipids is preferred, in which lipids are extracted into a bottom organic phase. Isolating the lipids from this lower fraction is tricky and can in part cause isolation of other phases as well. This leads to low purity of lipid samples which might negatively affect or hinder downstream lipid analysis. In addition, this method uses chloroform as the organic phase solvent, which is toxic and long-term exposure to it is hazardous. Assay Genie's Lipid Extraction Kit provides a chloroform free protocol of lipid isolation by organic extraction in a phase-free manner. The recovered organic phase can be dried and resuspended for further downstream lipid analysis. This protocol not only overcomes the difficulty of lower phase extraction and the dangers of using chloroform, but also provides for a very efficient and clean lipid extraction. Each kit provides sufficient reagents to isolate up to 50 lipid preparations based on a 25 µl sample size.

II. Applications:

- Lipid Extraction for downstream lipid analysis using Assay Kits (e.g. Cholesterol, Triglyceride etc.).
- Lipid Extraction for downstream lipid analysis with HPLC, MS and Lipidomics.
- Lipid Extraction for Screening, characterization and analysis of lipids under normal and disease conditions.

III. Sample Types:

- Serum, Plasma, Cultured Cells or Tissues.

IV. Kit Contents:

Components	BN00493	Cap Code	Part Number
Lipid Extraction Buffer	25 ml	NM	BN00493-1
Lipid Suspension Buffer	2.5 ml	Amber	BN00493-2

V. User Supplied Reagents & Equipment:

- Vacuum Concentrator
- Dounce homogenizer
- Vortex

VI. Storage and Reagents Preparation:

Store kit at RT, protected from light. Read entire protocol before performing the assay. Stable for one year.

VII. Lipid Extraction Protocol:

1. Sample Preparation:

Plasma: Collect blood with an anticoagulant such as citrate, EDTA, or heparin and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 min. Collect plasma supernatant without disturbing the white buffy layer. Samples can be extracted immediately or may be stored in aliquots at -80°C.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at RT for 30 min. Centrifuge at 2500 x g for 20 min. Collect the supernatant without disturbing the white buffy layer. Samples can be extracted immediately or may be stored in aliquots at -80°C.

Cultured Cells: Pellet 1-5 x 10^5 cells at 1000 x g for 5 min. Wash cells once with PBS and resuspend pellet in 25-50 µl PBS. Perform the extraction as described below.

Tissues: Carefully mince the tissue into small fragments with a scalpel/razor blade and weigh in a 50 ml conical tube. Take ~10-50 mg of wet weight tissue per sample for extraction. Perform the extraction as described below.

2. Lipid Extraction: The protocol below is for a 25 µl sample size. Number of preps per kit will decrease with increasing sample volumes.

a. Add 25 µl of sample (or 10-50 mg tissue) to the tube. Add 500 µl of Lipid Extraction Buffer to the sample. Quickly vortex for 1-2 min. on a tube shaker or vortexer. For tissues, we recommend to homogenize the tissue pieces in a Dounce homogenizer on ice. Spin the homogenate at 10,000 x g for 5 min. at 4°C and collect the supernatant.

b. Agitate the whole mixture for 15-20 min. on an orbital shaker at RT. Centrifuge the tube at 10,000 x g for 5 min. and carefully collect the supernatant containing lipids to a clean tube. Record the volume.

c. Leave this supernatant containing tube open and dry it in a vacuum concentrator or in a dry 37ºC incubator overnight (or until dry). A thin film can be seen after complete drying. The lipid sample can be further processed depending on the downstream application they are used for. If the downstream application is lipidomics, HPLC or MS, we recommend resuspending the lipid extract in an organic solvent such as butanol or cyclohexane. If the downstream application is analysis of lipids using Assay Kits (e.g. Cholesterol, Triglyceride etc.), we recommend resuspending the lipid extract in 50 µl of Lipid Suspension Buffer. Sonicate the lipid extract for 15-20 min. at 37°C.

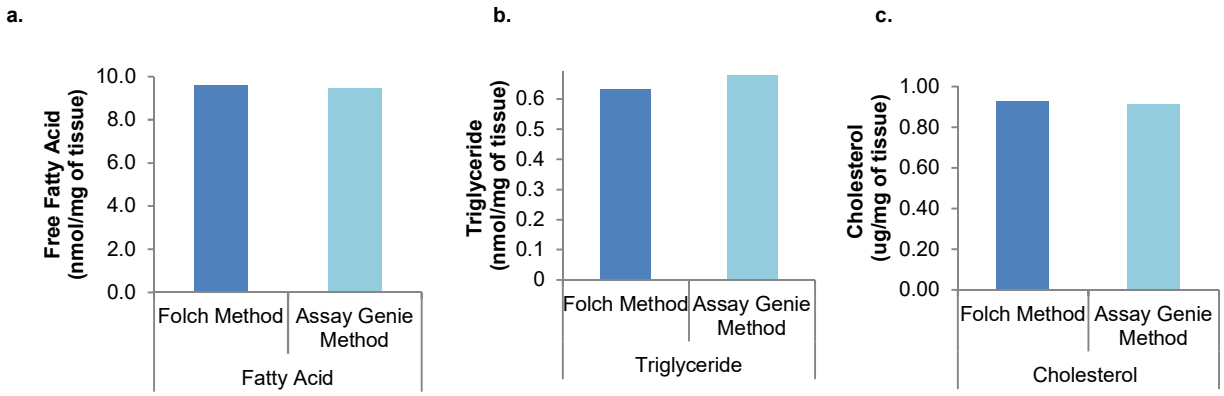

Figure 1: Analysis of Total Free Fatty Acid, Triglyceride and Cholesterol on Extracted Lipids using Rat Liver Tissue. Comparison of Total Free Fatty Acid (a), Triglyceride (b) and Cholesterol (c) using Folch Method and Assay Genie's Lipid Extraction Method.

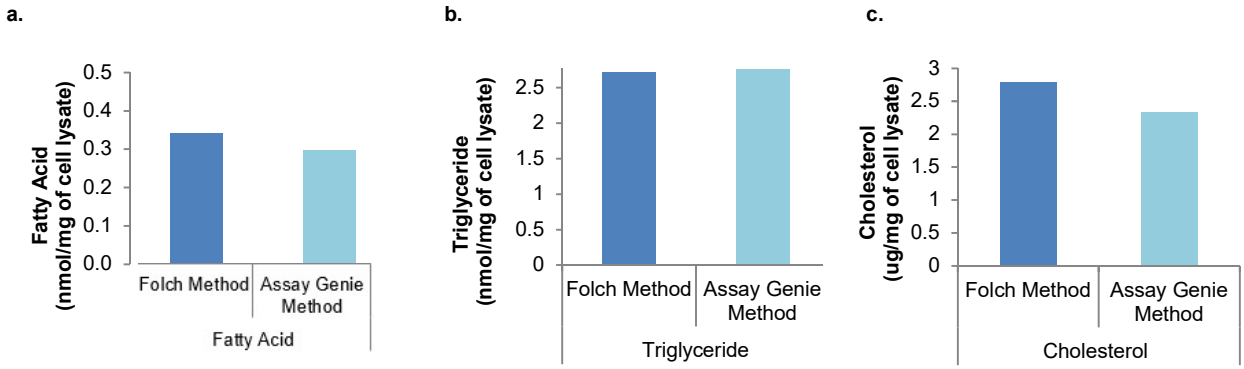

Figure 2: Analysis of Total Free Fatty Acid, Triglyceride and Cholesterol on Extracted Lipids using Adipocytes Cell Lysate. Comparison of Total Free Fatty Acid (a), Triglyceride (b) and Cholesterol (c) using Folch Method and Assay Genie's Lipid Extraction Method.

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