PI-0101

Lipid Extraction Kit

(Sufficient for 50 extractions, Store at room temperature)

Background Information:

One of the largest and most diverse subsets of molecules found in living systems is the lipids. They include the various fats, sterols, mono-, di- and triglycerides and phospholipids. While typically viewed as primarily a structural element of the cell (and subcellular) membranes, lipids are also important as a source of energy and in signaling. The lipids all share the property of having at least a portion of the molecule being hydrophobic. As a whole, the hydrophobicity of the lipids can vary over an extremely wide range although there is not a single physical measurement which can define it. One of the most common measures is the extent of partitioning between water and a hydrophobic liquid such as octanol. The most well-known methods to isolate lipids are the Folch and the Bligh and Dyer methods, both of which utilize chloroform and methanol in a ratio of 2:1 and 1:2, respectively to solubilize lipids. Starting in the 1960's environmental and other safety concerns has raised the awareness of the toxicity of the halocarbon solvents of which chloroform is a member. Much work was done to find other solvent mixtures which could uniformly extract all the lipids from biological materials. In 1978, Hara and Radin published a method using hexane and methanol which efficiently extracted almost all lipid types with high efficiency. AkrivisBio's Lipid Extraction Kit uses their formulation to provide an easy, efficient method of preparing lipid extracts on a small scale to be used for further analysis. One procedure using a 1 mg sample should recover up to 80 µg of lipid.

Kit Contents:

Lipid Extraction Solvent	25 ml	NM	PI-0101A
Sodium Sulfate Wash	10 ml	WM	PI-0101B
Lipid Suspension Agent	5 ml	NM	PI-0101C

User Supplied Reagents & Equipment:

- Dounce homogenizer
- Vacuum Concentrator

Storage and Handling:

Store the kit at room temperature. The included reagents are ready to use as supplied. Hexane in Lipid Extraction Solvent is quite volatile. The bottle should be kept tightly capped when not in use or hexane will evaporate preferentially, changing the solvent composition and reducing the extraction efficiency.

Lipid Extraction Protocol:

1. **Sample Preparation:**

Plasma and Serum: The Univ. of Cincinnati has a clear concise isolation protocol online: https://med.uc.edu/docs/default-source/mmpc-docs/serum_plasma-preparation.pdf. Use 25 µl as a sample.

Cells: Procedure varies with cell type. Centrifuge a suspension of HeLa cells at 100 X g, Jurkat cells at 200-300 X g for 5 minutes. Use 10^6 cells (~1 mg), pellet, carefully remove supernatant, resuspend in 1 ml PBS and repeat without disturbing the pellet.

Tissue: Take ~10 mg of wet weight tissue per sample for extraction. Keep moist and mince tissue with a razor blade while kept wet. Blot excess liquid, transfer to a previously weighed microcentrifuge tube and weigh to determine actual sample weight.

2. Lipid Extraction:

Add 200 µl Lipid Extraction Solvent. Vortex for 2 minutes. Minced tissue should then be homogenized using a Polytron or Dounce homogenizer.

a. Centrifuge at 10,000 x g for 5 min. at 4°C and transfer the supernatant to a clean microcentrifuge tube.

b. Add 100 µl Sodium Sulfate to the crude lipid extract and vortex for 1 minute to remove nonlipids. Transfer the clear upper layer to a fresh tube.

c. Dry extract under vacuum or a nitrogen stream until a thin film is seen and no liquid is left. Since many lipids are oxygen sensitive, it is not recommended to dry under conditions where air is in contact with the sample. The next step depends upon what the sample(s) are intended for. If chemical characterization by HPLC, MS or NMR is planned, keep dry at -20°C under N_2 or Argon until needed. Samples can then be dissolved in an organic solvent such as DMSO or hexane containing 0.02 mg/ml BHT. For analysis of the sample(s) by enzymatic assay for a particular analyte, add 50 µl Lipid Suspension Agent and vortex until completely dissolved. This can also be accomplished by carefully triturating the sample.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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