Extraction and analysis of liver lipids

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Abstract

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Protocol

Liver lipids extraction

Step 1.

1. Remove liver from -80°C freezer and place into cooler containing liquid nitrogen.

2. Place 50-100 mg liver onto glass plate and allow adipose tissue to thaw. Chop liver tissue with razor blade. The liver should still be in one piece after chopping.

3. Place 100 mg 5-alpha cholestane into 16x100mm tared glass screw top tubes. Dry off the solvent under N2 in the heating block.
4. Using a metal spatula, transfer the adipose tissue to the bottom of a tared 16x100mm glass screw top tube coated with 5-alpha cholestane. Do not scrape liver tissue along inside of the tube.
5. Weigh the tube plus liver tissue and record the liver tissue wet weight.
6. Add 3 ml 2:1 chloroform:methanol ($CHCl_3$:MeOH) and dislodge liver tissue from side of tube using a metal spatula.
7. Incubate the tube overnight at room temperature or in 55°C heating block for 1-2 hrs. When the liver tissue sinks to the bottom of the tube, the lipid is extracted.
8. Centrifuge tube at 1,500xg (2700 rpm) at room temperature for 10 min.
9. Transfer lipid extract to a new 16x100 mm glass screw top tube. Wash tube containing extracted liver tissue with 2 ml 2:1 $CHCl_3$:MeOH and centrifuge as above. Transfer wash to tube containing the lipid extract.
10. Dry down sample under N_2 in 55-60°C heating block.
11. Add 6 ml 2:1 $CHCl_3$:MeOH to tube and vortex. [Include a 15 ml glass graduated tube for calculation of bottom phase volume].
12. Add 1.2 ml 0.05% H_2SO_4 (sulfuric acid), cap tube, and vigorously vortex.
13. Centrifuge tube at 1,500xg (2700 rpm) at room temperature for 10 min. Record bottom phase volume of 15 ml graduated tube
14. Aspirate upper aqueous phase from tube.

For GC analysis

Step 2.

1. Transfer 1ml of bottom $CHCl_3$ into 16x100mm glass tube, dry off the solvent under liquid N_2 in the heating block.

2. Add 2 ml 95% EtOH and 200 ml of 50% of KOH to the tube
3. Cap tube and incubated at about 60°C for 3 hours with periodic vortexing.
4. Add 2 ml hexane and votex.
5. Add 2 ml of water and votex.
6. Centrifuge the tube at 2,700 rpm for 10 min at room temperatureTransfer the upper hexane phase to the clean 4 ml vial and dry off solvent using N_2 .

7. Resuspend extract in 250 ml of hexane and transfer to GC vial for analysis.

(If samples need to be sent out, dried it first before shipping out)

For enzymatic analysis

Step 3.

1. Using a glass volumetric pipet, transfer 1 ml of bottom phase to new 16x100 mm glass screw top tube.

2. Add 2 ml 1% Trition X100 dissolved in $CHCl_3$ and dry down sample under N_2 in 55-60°C heating block.

3. Add 1 ml water, cap, vortex, and place in 55-60°C heating block for 10 min. Vortex tubes at 5 min point of incubation.
4. Centrifuge tube at 1,500xg (2700 rpm) at room temperature for 5 min.

- If sample is clear then proceed to analysis of lipids using the enzymatic assays.

- If sample is cloudy, take a smaller volume of bottom phase (0.5-0.25 ml) and proceed through steps 15-17. Cloudiness indicates that the lipids were not completely dissolved by the Triton X100.

Enzymatic assays

Step 4.

- For the liver lipid analysis, use the standards containing Triton X100. Be sure that they are not plasma lipid standards.

Total cholesterol assay

Step 5.

- In 96 well plate, plate in duplicate 50 µl of each standard and sample.
- Add 150 µl total cholesterol reagent to each well (we are currently using Pointe Scientific cholesterol reagent). Incubate plate at room temperature (or 37°C depending upon reagent) for 1 hr and read the absorbance at 492 nm (or appropriate wavelength).

Free cholesterol assay

Step 6.

- In 96 well plate, plate in duplicate 50 µl of each standard and sample.
- Add 150 µl Free Cholesterol E (Wako) to each well. Incubate plate at room temperature for 1 hr and read the absorbance at 595 nm.

Triglyceride assay

Step 7.

- In 96 well plate, plate in duplicate 50 µl of each standard. In duplicate, plate 40 µl blank and 10 µl sample.
- Add 150 µl L-Type Triglycerides M reagent 1 (Wako) to each well and incubate plate at room temperature for 10 min.
- Add 50 µl L-Type Triglycerides M reagent 2 and incubate plate at room temperature for 1 hr. Read the absorbance at 595 nm.

Protein concentration of extracted liver

Step 8.

- Place uncapped tube containing extracted liver into 100°C oven for 20 min.
- Add 4 ml 1N NaOH and place capped tube into 100°C oven. Vortex tube every 30 min until liver is dissolved.
- Assay 5µl lysate in duplicate using a Lowry protein assay.