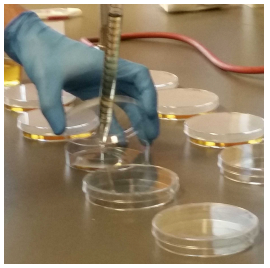
Feb 01, 2022 Version 2

Total Lipid Extraction from Baker's yeast (*Saccharomyces cerevisiae)* V.2



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Israel Olayide[1], Monica Rieth[2]

[1]St. Louis University Dept. of Chemistry; [2]Southern Illinois University-Edwardsville

Labyrieth

Monica Rieth
Southern Illinois University-Edwardsville

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol outlines a method for extracting total lipids from Baker's yeast, *Saccharomyces cerevisiae.* It has been adapted from Roy et al., *J. Lipid Res.* **2018.** doi: 10.1194/jlr.M088559.

Attachments

PDF

Extraction of lipids...

35KB

Guidelines

All culture ODs should be normalized for comparative analyses prior to the extraction. Weighed Eppendorf tubes to be used before getting started.
Extracted lipids can be stored at -20 degC

Troubleshooting

Safety warnings

chloroform and methanol should be used in a chemical hood

Day 1-3

3d

1 Sterilize the inoculating loop by dipping it in the ethanol and heating it for 30 second in the flame

1.1 Allow loop to cool the loop at least for 10 seconds

2 Streak plate from prepared glycerol stock (-80 °C) and spread on YPD agar plate

3 Allow plate to incubate for 2-3 days at 25-30 °C until colonies are 1-2mm in diameter

Day 4

1d

4 Inoculate 5 mL YPD with yeast colony from plate

5 Grow overnight at 30°C with shaking at 250 rpm

Day 5

1d

6 Check OD_{600} at UV-Vis spectrophotometer

7 Harvest the whole cells when OD_{600} is 1.5
Centrifuge at 1000 x g for 2 minutes or until clear to pellet the yeast

8 Pour off the supernatant without disturbing cell pellet

9 Wash the cells three times by resuspending pellets in 1ml PBS, centrifuge for at 500 x g for 3-5 mins.

10 After washing three times with PBS, resuspend in 1ml PBS and transfer this mixture to a 15 ml conical centrifuge tube.

11 Add 3.75 mL of chloroform/methanol (1:2) to the 1 mL of resuspended cells
Add 1.25 mL chloroform and 1.25 mL sterile water subsequently.

12 Vortex vigorously for 5 mins, and centrifuge at 150 x g for 5 mins at room temperature.

13 After centrifugation, a two-phase system is obtained: aqueous top phase and organic bottom phase which contains the lipids.

14 Carefully remove the top aqueous layer and the middle insoluble layers (precipitated proteins).

15 The organic bottom layer is dried using a speed vacuum or dried under a steady stream of nitrogen.

16 Record the weight of the dried sample by pre-weighing an Eppendorf tube or equivalent before or after drying the lipid.