

Extraction of Lipids from Yeast

Created on February 15, 2020 by Israel Olayide (reviewed by M. Rieth)

Adapted from: Roy et al., *J. Lipid Res.* **2018**. doi: 10.1194/jlr.M088559

Procedure

Day 1-3. Inoculation of Yeast

1. Sterilize the inoculating loop by dipping it in the ethanol and heating it for 30 second in the flame
2. Allow loop to cool the loop at least for 10 seconds
3. Streak plate from prepared glycerol stock (-80 °C) and spread on YPD agar plate
4. Incubate plate at 30°C until colonies 1-2 mm in diameter have formed (2-3 days)

Day 4. Liquid culture

1. Inoculate 5 ml YPD with yeast colony from plate
2. Grow overnight at 30°C

Day 5. Extraction

1. Check OD₆₀₀ at UV-Vis spectrophotometer
2. Harvest the whole cells when OD₆₀₀ is 1.5
3. Centrifuge at 1000 x g for 2 minutes or until clear to pellet the yeast
4. Pour off the supernatant without disturbing cell pellet
5. Wash the cells three times by resuspending pellets in 1ml PBS, centrifuge for at 500 x g for 3-5 mins.
6. After washing three times with PBS, resuspend in 1ml PBS and transfer this mixture to a 15 ml conical centrifuge tube.
7. Add 3.75 ml of chloroform/methanol (1:2) to the 1 ml of cell sample
8. Vortex well for 15 mins and incubate on ice for 5 mins.
9. Add 1.25 ml chloroform and 1.25 ml sterile water subsequently.
10. Vortex vigorously for 5 mins, and centrifuge at 150 x g for 5 mins at room temperature.
11. After centrifugation, a two-phase system is obtained: aqueous top phase and organic bottom phase which contains the lipids.
12. Carefully remove the top aqueous layer and the middle insoluble layers (precipitated proteins).
13. The organic bottom layer is dried using a speed vacuum or under a steady stream of nitrogen.
14. Record the weight of the dried sample by pre-weighing an Eppendorf tube or equivalent before and after drying the lipid.

*Note: if comparing cultures grown under different conditions, all cultures should be normalized to the same OD prior to pelleting and lipid extraction and before sending for lipidomic analysis