Lipid extractions from labyrinthulomycetes

Laura Halligan,Jackie Collier

Abstract

This protocol is a two-part lipid extraction for labyrinthulomycetes. The only strain we have tried this protocol on so far is *Aurantiochytrium limacinum* ATCC MYA-1381.

This protocol was modified from: Higgins, B.T., A. Thornton-Dunwoody, J.M. Labavitch, J.S. VanderGheynst. 2014. Microplate assay for quantitation of neutral lipids in extracts from microalgae. Anal. Biochem. 465: 81-89.

Citation: Laura Halligan,Jackie Collier Lipid extractions from labyrinthulomycetes . **protocols.io** dx.doi.org/10.17504/protocols.io.qbddsi6
Published: 31 May 2018

Protocol

Preweigh and label new, empty tubes

Step 1.

Weigh a 2.0 ml polypropylene microcentrifuge tube, record weight.

Label 2 - 15 ml polypropylene centrifuge tubes 'A' and 'B'.

Will also need another 2 - 15 ml polypropylene centrifuge tubes for the steps 8-11.

'A' is the first lipid extraction so all of the labeled tubes for the first extraction are labeled 'A'.

'B' is the second lipid extraction so all of the labeled tubes for the second extraction are labeled 'B'.

Collect cells

Step 2.

Add 2.0 ml of cells to preweighed 2.0 ml microcentrifuge tube and spin down to create pellet. Remove supernatant.

*We have tried this protocol using freeze dried cells and there does not seem to be a difference in the amount of lipids extracted. The fresh cells are easier to homogenize. From our experience, the total lipids extracted were an average of 12% of wet weight and an average of 34% of dry weight.

Step 3.

Weigh the 2.0 ml microcentrifuge tube containing the fresh cell pellet and record weight.

*The difference between the weight of the 2.0 ml microcentrifuge tube with the fresh pellet and the empty, starting weight of the tube is an estimate of cell mass.

Lipid extraction

Step 4.

Add 1.0 ml Folch solvent (2:1 chloroform/methanol) to the pellet in the 2.0 ml microcentrifuge tube.

Step 5.

Manually homogenize the pellet in the 2.0 ml microcentrifuge tube with a pestle (Fisher Scientific catalog no. 12-141-363).

*We have also tried using 0.5 mm zirconia-silica beads instead of a pestle or in combination with a pestle and there does not seem to be a difference in the amount of lipids extracted.

Step 6.

Add an additional 0.5 ml Folch solvent to the pellet in the 2.0 ml microcentrifuge tube. (Total volume of Folch is 1.5 ml).

Step 7.

Spin down the pellet in the 2.0 ml microcentrifuge tube at 13.2 rpm for 5 minutes.

Step 8.

Transfer supernatant to a 15 ml polypropylene centrifuge tube.

Step 9.

Add 4.5 ml Folch solvent to the supernatant in the 15 ml centrifuge tube.

Step 10.

Add 1.2 ml 0.9% NaCl to the mixture in the 15 ml centrifuge tube.

*Shake the the Folch solvent - NaCl mixture before centrifuging.

Step 11.

Centrifuge the 15 ml centrifuge tube at 6,000 g for 6 minutes.

Step 12.

Transfer the chlorofom (bottom) phase to the labeled ('A' or 'B') - 15 ml centrifuge tube.

*Be sure to not transfer any of the debris floating at the top of the chlorofom phase and any of the aqueous (top) phase into to the labeled - 15 ml centrifuge tube.

Second lipid extraction

Step 13.

On what is left of the cell pellet in the 2.0 ml microcentrifuge tube, do a second 'B' lipid extraction. Redo steps 4-12.

Dry extracts

Step 14.

Setup the labeled - 15 ml centrifuge tubes containing the chloroform phase (and lipids) under a filtered air stream. The time it takes to dry depends on how much fluid there is.

*From our experience, dried lipids from the first 'A' extraction are primarily yellow/orange and dried lipids from the second 'B' extraction are primarily white/cloudy.

Step 15.

Label 2 - 0.35 ml aluminum micro weighing dishes (VWR catalog no. 12577-062) 'A' and 'B' and once ink has dried (if the ink has not fully dried the weight of the dish will not be correct), record their weights using a milligram balance.

*We found that weighing the lipid extracts in aluminum micro weighing dish on a milligram balance gave consistent weights as opposed to weighing the extracts in a polypropylene microcentrifuge tube on an analytical balance.

Transfer lipid extracts to aluminum micro weighing dishes

Step 16.

When lipid extracts in the 15 ml centrifuge tubes appear mostly dried, we have been transferring the extracts into a 1.5 ml microcentrifuge tube. Once the extracts in the 1.5 ml tube appear mostly dried, we transfer them to the 0.35 ml aluminum micro weighing dishes. We are not sure yet whether it is better to do an intermediate transfer with the 1.5 ml tubes to the 0.35 ml aluminum dishes or to transfer straight from the 15 ml tubes to the 0.35 ml aluminum dishes.

When transferring lipid extracts 'A' and 'B' from the 1.5 ml tubes to the 0.35 ml aluminum dishes, start with a larger volume of Folch solvent (300 ul) to redissolve the extracts and rinse the tubes. After the first rinse or two, use smaller volumes of Folch solvent (100 ul) to rinse the tubes and transfer the lipids. Be sure all the lipids have been transferred and the tubes look clean.

*It is important to use a larger volume of Folch for the first rinse to ensure the lipids dissolve otherwise the lipids will get stuck in the pipette tip. If the Folch reagent in the 0.35 ml aluminum dishes does not evaporate fast enough in between washes, let it evaporate a little before you do the next wash so you do not over flow the aluminum dish.

Dry extracts

Step 17.

Leave the 0.35 ml aluminum dishes with the lipid extracts to dry under a filtered air stream.

*The dishes were usually left under the filtered air stream for 2 days. We have not yet figured out how long it takes to dry to constant weight.

Weigh extracts

Step 18.

Once the lipid extracts have fully dried in the 0.35 ml aluminum dishes, weigh them using a milligram balance.

*Again, dried lipids from the first 'A' extraction are primarily yellow/orange and dried lipids from the second 'B' extraction are primarily white/cloudy. From our experience, an average of 85% of lipids are collected in the first 'A' extraction and an average of 15% of lipids are collected in the second 'B' extraction.