

Isolation of Mitochondria using the Thermo Scientific Fiberlite F21S-8x50y Carbon Fiber Rotor in Thermo Scientific Sorvall Superspeed Centrifuges

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KEY WORDS

- Mitochondria Isolation
- Discontinuous Sucrose Gradient
- Large Volume Pelleting
- Yeast Pelleting
- Superspeed Centrifuges
- Carbon Fiber Rotors

Introduction

Mitochondrial isolations are required for studies of a wide range of biological questions, especially in bioenergetics. The purity of the mitochondrial preparation is extremely important when studying the functional assembly of mitochondrial DNA. This procedure describes the isolation of mitochondria from large volume cultures of the yeast *Saccharomyces cerevisiae* using Thermo Scientific Fiberlite carbon fiber rotors and Thermo Scientific Sorvall superspeed centrifuges. In addition, this procedure can be readily adapted to other cell types, including bacteria, insects, or mammalian cells.

The following protocols were developed based on References 1–4.

Procedure

PROTOCOL 1: Pelleting the Mitochondria

1. Grow a pre-culture of the yeast *Saccharomyces cerevisiae* at 30°C in Difco YM broth overnight.
2. Transfer 8 mL of pre-culture into four flasks containing 2 L of the above medium and grow cells at 30°C overnight to OD600.
3. Place 1L of the yeast culture into a Thermo Scientific Nalgene 1L superspeed bottle for pelleting.
4. Pellet the cells at 2000 x g (3,000 rpm) for 15 min in a large-volume Fiberlite™ F8S-6x1000y or F9S-4x1000y rotor in a Sorvall® Evolution™ RC or Sorvall® RC-6™ Plus centrifuge.
5. Wash the cells with cold distilled water and spin again in the large volume Fiberlite rotor at 2,000 x g (3,000 rpm) for 15 min.
6. Re-suspend the pelleted cells in 0.1 M Tris-HCl, 1% 2-mercaptoethanol (pH 9.3), and incubate at 32°C for 10 min.
7. Re-pellet cells using 0.01 M Tris-HCl, 0.5 M KCl (pH 7.0).
8. Re-suspend the cells in 0.01 M citrate-phosphate buffer (pH 5.8) 1 mM EDTA, 1.35 M sorbitol.
9. Add 1 mg/mL Zymolyase 20T (ICN Biomedicals) and shake at low speed for 90 min at 32°C in N₂ atmosphere to digest the cell wall for spheroplast release.
10. Centrifuge the spheroplast suspension to pellet the spheroplasts in the F21S-8 x 50y rotor with Bioseal technology for 10 min at approximately 3,000 x g (5,000 rpm) at 4°C. Discard the supernatant.
11. Re-suspend the pellet and sediment it twice with 40 mL of 0.01 M Tris-maleate, 0.75 sorbitol, 0.4 M mannitol 2 mM EDTA, 0.1% BSA, pH 6.8.
12. Re-suspend the final pellet in 80 mL of 0.01 M Tris-maleate, 0.6 M mannitol, 2 mM EDTA, 0.2%



Sorvall superspeed centrifuges, Fiberlite large volume carbon fiber rotors and Nalgene 1L superspeed centrifuge bottle is a powerful system solution designed to accelerate your sample processing.



The Fiberlite F21S-8x50y fixed-angle carbon rotor

13. Centrifuge aliquots at approximately 1,000 x g (3,000 rpm) for 10 min. Collect the supernatant. Repeat step 10 and combine supernatants.
14. Centrifuge at approximately 17,000 x g (12,000 rpm) for 10 min at 4°C in the F21S- 8 x 50y rotor with Bioseal technology. Collect the mitochondrial pellet.

PROTOCOL 2: Sucrose Gradient Purification of Mitochondria in the Fiberlite F21S-8 x 50y rotor with Bioseal technology.

(*The entire purification is carried out at 4°C.)

1. Prepare a discontinuous sucrose gradient (30% w/w, 40% w/w, 50% w/w, 60% w/w), containing 1 mM EDTA, 0.1% BSA, 10 mM Tris-HCl (pH 7.5) using cold sucrose concentrations. (5)
2. Re-suspend the mitochondrial pellet in about 0.5 mL of 0.5 M sucrose solution to be used for each centrifuge tube with the discontinuous gradient.
3. Gently layer the mitochondrial suspension on the sucrose gradient and centrifuge for 2.5 h in the F21S-8x50y rotor at 47,000 x g (20,000 rpm). The intact mitochondria form a brown band at 1.19 g/mL sucrose density (approximately the center of the tube). One or more impurity bands may be seen below the mitochondria band.
4. Remove the mitochondria band and dilute it with 2 volumes of 1 mM EDTA 10 mM Tris -HCl (pH 7.4). Pellet the mitochondria by centrifugation at approximately 26,000 x g (15,000 rpm) for 10 min. Collect the mitochondrial pellet and re-suspend in pH 7.5 buffer. Add 1% SDS and 50 mg/mL proteinase K. Incubate for 3 to 3.5 h at 37°C with slow shaking. Solution can be stored for use in further studies.

Conclusion

This technical note describes a process for the purification of mitochondria from yeast cells using reliable Thermo Scientific Fiberlite carbon fiber rotors with Bioseal technology and dependable leakproof Nalgene large volume superspeed bottles. This combination offers the total solution for efficient and safe processing of all your samples. This protocol can be adapted to retrieve mitochondria from other cell types such as bacterial, mammalian, or insect cells.

Rotors featuring Bioseal technology have been rigidly tested for microbiological containment by the Public Health Laboratory Service, Centre for Applied Microbiological Research, Porton Down, UK, and shown to be suitable for use with materials up to ACDP Category 3 as categorized by the Advisory Committee on Dangerous Pathogens.

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