Mitochondria Isolation from VERO E6 Cells

RS18-0280A

Materials

- RED Bead Lysis Kit
- 1X PBS
- Hypotonic Buffer (IB 0.1x: 3.5 mM Tris-HCl, pH 7.8, 2.5 mM NaCl, 0.5 mM MgCl2)
- Hypertonic Buffer (IB 10: 0.35 M Tris-HCl, pH 7.8, 0.25 M NaCl, 50 mM MgCl2)
- Wash Buffer A (0.32 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4)
- Extraction Buffer (20mM Tris-HCl, pH 7.8, 20 mM EDTA, 150 mM NaCl, 1% SDS)
- Proteinase K
- Phenol-Chloroform-Isoamyl Alcohol (25:24:1)
- 0.2 M Ammonium Acetate
- Ethanol
- Bullet Blender Storm Pro

Methods

Homogenization

1. Harvest and pellet 2.5 million cells in microcentrifuge tubes by centrifuging at 600 x g for 5 minutes. Wash twice with 1X PBS and then remove the supernatant.
2. Place the cells pellet on ice and add 600 µL of hypotonic buffer to the tube.
3. Resuspend the cells by pipetting up and down and then transfer the entire volume into a RED lysis kit tube.
4. Set the Bullet Blender to speed 8 and homogenize the cells for 1 minute.
5. Using a pipette, remove approximately 600 µL of the homogenate and transfer it into a new microcentrifuge tube.
6. Immediately after transfer, add 60 µL of hypertonic buffer to the lysis tube.
7. Centrifuge the homogenate at 1200 x g for 3 minutes at 4°C to pellet unbroken cells, debris and nuclei.
   Note: The supernatant contains the mitochondria and will be cloudy. A small cell/debris pellet signifies that homogenization of cells was thorough.
8. After centrifugation, collect the supernatant into a new tube and centrifuge again at the same condition to ensure a complete elimination of heavy contaminants.
9. Collect the supernatant containing the mitochondria into a new tube.
10. Centrifuge the sample at 15,000 x g for 4 minutes at 4°C to pellet the mitochondria.
11. Remove the supernatant and wash the mitochondria pellet with 100 µL of Wash Buffer A.
12. Pellet the mitochondria again and repeat the wash step once more with Wash Buffer A.
13. Pellet the mitochondria once more and remove the supernatant.
14. If immediately processing mitochondria for DNA extraction, continue on to the Extraction procedure. If storing mitochondria for later use, equilibrate the mitochondria pellet by resuspending in the Wash Buffer A and store at -80°C until use.

Mitochondria Isolation from VERO E6 Cells

RS18-0280A

Mitochondria DNA Extraction

1. To the mitochondria pellet, add 500 µL of ice-cold extraction buffer and 20 µL of proteinase K and resuspend the pellet.
2. Incubate the mitochondria suspension at 55°C for 20 minutes to lyse the membranes.
3. To purify the DNA after incubation, add 500 µL of phenol-chloroform-isoamyl alcohol and mix thoroughly.
4. Centrifuge the samples at 12,000 x g for 5 minutes.
5. Using a pipette, transfer the aqueous phase (top layer) to a new tube.
6. To precipitate the DNA, add 12 µL 0.2 M ammonium acetate and 700 µL of cold ethanol. Freeze immediately on dry ice for 1 hour.
7. Continue to precipitate the DNA overnight at -20°C.
8. Centrifuge the sample at 12,000 x g for 10 minutes at 4°C to pellet the mtDNA.
9. Wash the mtDNA pellet twice with 70% ethanol and finally, resuspend the mtDNA in desired media.
10. Analyze DNA quality and yield. Isolated DNA can be stored at -20°C.