

Isolation of Mitochondria from Human Fibroblasts

- 2x 150mm plates of cells
- Wash cells 2x with PBS
- Scrape cells (with 1 mL PBS) and collect them into 1.5mL microcentrifuge tubes
- Spin at maximum speed for 3 minutes to pellet, remove supernatant and store at -80°C
- Thaw cell pellet on ice and transfer to homogenizer with 5mL of Isolation buffer
- Homogenize cells on ice, 10 strokes
- Transfer homogenized cells to 4 microcentrifuge tubes (~1.2mL per tube)
- Spin at 600g at 4°C for 10 minutes
- Transfer supernatant to new microcentrifuge tubes
- Spin at 10000g at 4°C for 10 minutes
- Resuspend mito pellets in PBS (15µL per pellet)

Isolation buffer:

- 0.25M Sucrose
- 10mM Tris, pH 7.5
- 1mM EDTA

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