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Mitochondrial isolation from cultured fibroblasts

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the procedure used by the Seifert Lab to isolate mitochondria from cultured fibroblasts.

Protocol materials

EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**ED-1KG

Deionized water

0.5 M EDTA **Fisher Scientific Catalog #**50-983-251

1 Dislodge cultured fibroblasts with trypsin

2 Spin cell down at 1500 rpm, 00:05:00

3 Discard supernatant and keep pellet On ice

4 Resuspend pellet in 1 mL Mitochondria Isolation Buffer (MIB; [M] 215 millimolar (mM) mannitol, [M] 75 millimolar (mM) sucrose, 0.1% BSA, [M] 20 millimolar (mM) HEPES, and [M] 1 millimolar (mM) EGTA, adjusted to pH 7.2 with KOH)

5 Spin suspension at 400 rcf, 00:05:00

6 Discard supernatant

7 Resuspend pellet in 450 µL
EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**ED-1KG

8 Break cells with a Nitrogen cell disruptor at 1250 Bar for 00:10:00

9 Pass lysates through a 26-gauge syringe topped with 1.5 mL MIB by spinning at 1300 x g, 4°C, 00:03:00

10 Repeat Step 9

11 Transfer supernatant to a new tube and spin 1300 x g, 4°C, 00:10:00

12 Apply supernatant to Ficoll gradient (25 mL STE [8.55 g sucrose, 32 µL 0.5 M EDTA **Fisher Scientific Catalog #**50-983-251 , 80 µL 1 M Tris, up to 25 mL with Deionized water] and Ficoll stock [10 mL STE , 17.5 mL 20% Ficoll])

13 Spin at 3200 rpm, 00:30:00

14 Discard supernatant

15 Resuspend pellet in 1 mL MIB

16 Spin at 13000 rcf, 00:10:00

17 Discard supernatant

18 Resuspend pellet (purified mitochondria) in MIB