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Subcellular Fractionation

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We use this protocol and it's working

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Abstract

To isolate total cell lysate into cytosolic and mitochondrial, soluble vs insoluble fractions.

Materials

- Cell scraper
- 1X PBS
- Glass pestle
- Isolation medium (250 mm sucrose, 1 mm EDTA and 10 mm Tris–MOPS, pH 7.4)
- Microcentrifuge tubes
- Ultracentrifuge
- Triton X-100
- Ice
- SDS buffer (50 mm Tris, 2% SDS, pH 7.6)

1 Harvest cells with a scraper.

2 Resuspend PBS and centrifuge at 500g for 5 min.

3 Homogenize using a glass pestle in isolation medium.

4 Centrifuge at 600 g for 10 min at 4°C.

5 Transfer the supernatant to a new microcentrifuge tube and centrifuge at 9,000 g for 10 min at 4°C.

6 Transfer the supernatant (cytosolic fraction) to a new microcentrifuge tube.

7 Resuspend the pellet with isolation medium.

8 Centrifuge at 9,000 g for 10 min at 4°C.

9 Repeat step 7 and 8 for a total of 3 times.

10 The final pellet is collected as the mitochondrial fraction.

11 Add Triton X-100 to both cytosolic and mitochondrial fractions to a final concentration of 1%.

12 Incubate mixture from both fractions on ice for 30 min.

13 Centrifuge at 20,000 g for 10 min at 4°C.

14 Transfer the supernatant (soluble fraction) to a new microcentrifuge tube.

15 Incubate the pellet in SDS buffer for 30 min.

16 Centrifuge at 20,000g for 10 min at room temperature.

17 The final solution is the insoluble fraction.