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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.