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Mitochondrial isolation

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Protocol status: Working

We use this protocol and it's working

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Abstract

Mitochondrial isolation

Mitochondrial isolation

- 1 Harvest and pellet 10×10^6 cells.
- 2 Incubate the pellet on ice for 10 minutes with 1 mL of lysis buffer supplemented with a protease inhibitor.
- 3 Centrifuge the suspension at $1000 \times g$ for 10 minutes at 4°C .
- 4 Retain half of the supernatant as the total cellular fraction.
- 5 Subject the remaining supernatant to three additional rounds of centrifugation at $1000 \times g$ for 10 minutes at 4°C to obtain the cleaned supernatant as the cytosolic fraction.
- 6 Resuspend the pellet from the first centrifugation step in 1.5 mL of disruption buffer supplemented with a protease inhibitor.
- 7 Mechanically disrupt the suspension by passing it through a 26-gauge needle ten times on ice.
- 8 Centrifuge the disrupted suspension at $1000 \times g$ for 10 minutes at 4°C and discard the pellet.
- 9 Centrifuge the resulting suspension at $14000 \times g$ for 30 minutes at 4°C .
- 10 Resuspend the final pellet enriched with mitochondria in 50 μL of Mitochondria Storage Buffer supplemented with a protease inhibitor.
- 11 Measure the protein concentration in all fractions using the PierceTM BCA Protein Assay Kit (Cat. number 23225, Pierce, Thermo Fisher Scientific).
- 12 Use 20 μg of protein for immunoblotting and 30 μg for measurement of protein aggregation.