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Mitochondrial isolation from HeLa cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

Mitochondrial isolation and quantification from HeLa cells.



Mitochondrial isolation

40m

- 1 Thaw cell pellets that have been frozen at $-80\text{ }^{\circ}\text{C}$ for at least 00:15:00, on ice. 15m
- 2 Aliquot out an amount of Solution B (220 mM mannitol, 70 mM sucrose, 1 mM EDTA, 20 mM HEPES-KOH pH 7.6) assuming for 5 mL of Solution B per sample (if samples have been collected from 10cm plates), and add PMSF at a final concentration of 0.5 mM . Keep the Solution B on ice at all times.
- 3 Resuspend the first sample in 3 mL of Solution B (+PMSF), and homogenize with 30 strokes using a Dounce hand-held glass homogenizer (7 mL capacity)
- 4 Move the homogenate back to the original tube, and wash the homogenizer using distilled water.
- 5 Repeat steps 3 – 4 for each sample.
- 6 After all samples have been homogenised, spin all samples for 00:05:00 at $4\text{ }^{\circ}\text{C}$ and 800 rcf . 5m
- 7 Transfer the supernatant to microfuge tubes (will need 3 microfuge tubes per sample if starting with 3 mL), being careful to not touch the pellet.
- 8 Spin the supernatant for 00:10:00 at $4\text{ }^{\circ}\text{C}$ and 10000 rcf . The resulting pellet will contain mitochondria. 10m
- 9 Carefully aspirate the supernatant from each tube.
- 10 Collate the individual tubes for each sample into one microfuge tube, by resuspending mitochondrial pellets in 200 μL of Solution B and pooling together into one tube.



Rinse each tube with another  200 μL of Solution B, and combine these two volumes together into the one tube.

11 Spin the resuspended mitochondria for  00:10:00 at  4 °C and  10000 rcf .

10m

12 Carefully aspirate the supernatant from each tube.

13 Resuspend each sample in ~300-400 μL of Sucrose Storage Buffer ( 0.5 Mass Percent sucrose,  10 millimolar (mM) HEPES-KOH pH 7.6) (depending on the size of the mitochondrial pellet). Aliquot  15 μL into a separate tube (to be used for protein quantification), and then split the sample into three separate tubes for storage (this is to allow the samples to be used more times without being freeze-thawed excessively). Store mitochondria in Sucrose Storage Buffer at  -80 °C . When required, thaw the samples on ice before use.

Quantification of mitochondrial samples

20m

14 Centrifuge the  15 μL aliquot taken from each sample at  4 °C for  00:10:00 at  10000 rcf .

10m

15 Carefully aspirate the supernatant from each tube.

16 Add  5 μL of 1x LDS lysis buffer (ThermoFisher) to each sample.

17 Boil samples at  99 °C shaking at maximum speed for  00:10:00 .

10m

18 Let samples cool to  Room temperature , quickly centrifuge to collate the liquid at the bottom of the tube, and vortex each sample to ensure it is homogenous.

19 Blanking with the 1x LDS lysis buffer, measure the protein level spectroscopically using an A280 measurement.



- 20 Divide each concentration by 3, and that will represent the concentration of mitochondria in each sample.