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Mitochondrial isolation

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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 deg C for at least 00:15:00, on ice — 15m

2 Aliquot out an amount of Solution B 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 deg C and 800x 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 deg C and 10,000x 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 uL of Solution B, and combine these two volumes together into the one tube.

11 Spin the resuspended mitochondria for 00:10:00 at 4 deg C and 10,000x rcf. 10m

12 Carefully aspirate the supernatant from each tube.

13 Resuspend each sample in ~300-400 uL of Sucrose Storage Buffer (depending on the size of the mitochondrial pellet). Aliquot 15 uL 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 deg 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 deg C for 00:10:00 at 10,000x rcf. 10m

15 Carefully aspirate the supernatant from each tube.

16 Add 5 µL of LDS lysis buffer to each sample.

17 Boil samples at 99 deg 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 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.