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Preparation of soluble and insoluble mitochondrial protein fractions for immunoblotting

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We use this protocol and it's working

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Abstract

Preparation of soluble and insoluble mitochondrial protein fractions from HeLa cells for immunoblotting.

Troubleshooting

1

Note

All steps are performed on ice, unless specified

Thaw mitochondrial stocks on ice, and take two aliquots of 15 µg of mitochondria each for each sample (one aliquot will be the 'total' sample fraction, and one aliquot will represent the 'fractionation' sample).

2 Centrifuge samples at 10000 rcf for 00:10:00 at 4 °C, and carefully aspirate the supernatant. 10m

3 Add 15 µL of ice cold 0.5% v/v TX-100 in PBS, vortex each sample for ~5 seconds, and leave samples to incubate on ice for 00:15:00. At the conclusion of the 15 min lysis incubation, vortex each sample for ~5 sec again. 15m

4 Set aside one fraction from each sample for processing downstream (referred to as the 'total' fraction).

5 Centrifuge the remaining tube of each sample at 12000 rcf for 00:10:00 at 4 °C 10m

6 Using a pipette, carefully remove 14.24 µL of the supernatant (representing 95% of the lysis volume) and place this sample into a clean microfuge tube on ice, being sure not to disturb any pellets that have formed on the bottom of the tube. The volume removed represents the 'soluble' protein fraction. The tube containing the pellet represents the 'insoluble' protein fraction.

7 Add 14.24 µL of 0.5% TX-100 in PBS to each insoluble protein fraction by pipetting on the side of the tube. Gently flick each tube to rinse the sides of the tube.

8 Centrifuge the insoluble protein fractions at 12000 rcf for 00:10:00 at 4 °C. 10m

9 Using a pipette, carefully remove 14.24 µL of the supernatant from the insoluble protein fraction, and place into a clean microfuge tube (which will function as the waste collection tube for all samples).

10 Repeat steps 7 – 9, which will total 2 washes

11 Repeat step 7. You should now have three tubes for each sample (representing the total, soluble and insoluble protein fractions), and one waste tube.

12 Bring all samples to room temperature. To the total and insoluble samples, add 5 µL of 4x SDS loading dye (4x: 20% w/v SDS, 400 mM DTT, 40% v/v glycerol, 200 mM Tris-Cl pH 6.8). To the soluble fraction, add 4.75 µL of 4x SDS loading dye.

> Note
>
> The precise quantities of loading dye added are very important, as they influence how the SDS-PAGE gel will run later on.

13 Vortex each sample for ~3 seconds

14 Boil all samples at 99 °C for 00:10:00 shaking at maximum speed. 10m

15 Let samples cool to Room temperature, and centrifuge quickly to bring all liquid to the bottom of the tubes. Vortex the samples again for ~3 sec to ensure homogeneity.

16 Sonicate all samples in a waterbath sonicator set to 21 °C water temperature for 00:02:00. 2m

17 Remove all samples from the sonicator, vortex samples for ~3 sec to mix.

18 Load 19 µL from each sample on an SDS-PAGE gel and proceed to run and transfer the gel as per a standard immunoblotting protocol.