Preparing mitochondrial samples for immunoblot analysis

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Protocol for preparation of mitochondrial samples for immunoblot analysis.

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1 Thaw mitochondrial stocks on ice, and aliquot out the desired amount of mitochondria.

2 Centrifuge each aliquot for 00:10:00 10000 x g, 4°C 10m

3 Carefully aspirate the supernatant from each sample.

4 Add a volume of 1x SDS sample buffer (5% w/v SDS, 10% v/v glycerol, 100 mM DTT, 50 mM Tris-Cl pH 6.8) equal to the amount of mitochondria (in ug) to each sample. Eg. If each sample contains 20 µg of mitochondria, add 20 µL of 1x SDS sample buffer.

5 Vortex samples for ~5 seconds to mix, and then boil at 99 °C with shaking at max speed for 00:10:00 . 10m

6 Allow samples to cool to room temperature, quickly centrifuge to collect liquid to the bottom of the tube, and vortex for ~3 seconds to ensure the samples are homogenous.

7 Samples can now be directly loaded onto an SDS-PAGE gel, or stored at -20 °C for later use.