Quick-Start Protocol February 2016

Qproteome® Mitochondria Isolation Kit

Lysis Buffer of the Qproteome Mitochondria Isolation Kit (cat. no. 37612) should be stored at –30 to –15°C upon arrival. All other buffers and Protease Inhibitor Solution (100x) should be stored at 2–8°C.

Further information

- *Qproteome Mitochondria Isolation Handbook*: **www.qiagen.com/HB-0870**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for standard preparation of mitochondria from eukaryotic cell lysates. For high-purity preparation and preparation from tissues, refer to the handbook.
- All steps are performed at 4°C. Use pre-cooled buffers. Separated protein fractions should be snap-frozen in liquid nitrogen and stored at –80°C.
- For downstream applications (e.g., SDS-PAGE analysis), elution fractions should be pooled and concentrated (e.g., by acetone precipitation).
- Immediately before use, supplement Lysis Buffer and Disruption Buffer with 1/100 volume of Protease Inhibitor Solution (100x) (i.e., if using 2 ml Disruption Buffer, add 20 µl Protease Inhibitor Solution [100x]).

1. Transfer a cell suspension containing approximately 5 x 10^6 to 2 x 10^7 cells into a 15 ml conical tube and centrifuge at 500 x *g* for 10 min at 4°C. Carefully remove and discard the supernatant.
2. Wash the cells using 1 ml 0.9% sodium chloride solution.

3. Resuspend the cell pellet in 1 ml (<1 x 10^7 cells) or 2 ml (≥1 x 10^7 cells) ice-cold Lysis Buffer by pipetting up and down using a 1 ml pipet tip. Incubate for 10 min at 4°C on an end-over-end shaker.
4. Centrifuge the lysate at 1000 x *g* for 10 min at 4°C.
5. Carefully remove the supernatant.

   This fraction primarily contains cytosolic proteins.
6. Resuspend the cell pellet in 1.5 ml ice-cold Disruption Buffer by pipetting up and down using a 1 ml pipet tip. Complete cell disruption by using a blunt-ended needle and a syringe (not provided). Draw the lysate slowly into the syringe and eject with one stroke. Repeat 10 times. Alternatively, disrupt cells using a Dounce or Potter homogenizer.
7. Centrifuge the lysate at 1000 x *g* for 10 min at 4°C and carefully transfer the supernatant to a clean 1.5 ml tube.

   The pellet contains nuclei, cell debris and unbroken cells. If desired, proteins can be re-extracted from the cell pellet by repeating steps 6 and *7* using 500 µl ice-cold Disruption Buffer. The supernatants from each extraction should be combined before the next step.
8. Centrifuge the supernatant(s) from step *7* at 6000 x *g* for 10 min at 4°C.
9. Carefully remove the supernatant.

   The pellet contains mitochondria. The supernatant constitutes the microsomal fraction.
10. Wash the mitochondrial pellet from step 9 with 1 ml Mitochondria Storage Buffer by carefully pipetting up and down using a 1 ml pipet tip. Centrifuge at 6000 x *g* for 20 min at 4°C.
11. Resuspend the mitochondrial pellet in Mitochondria Storage Buffer or a buffer of choice for further analysis.

Scan QR code for handbook.