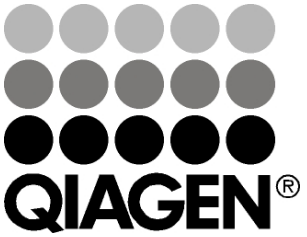
Second Edition July 2011

Qproteome® Mitochondria Isolation Handbook

For purification of mitochondria from eukaryotic cells and tissues



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Contents

Kit Contents

Qproteome Mitochondria Isolation Kit	**Cat. no. 37612**
Number of preps	**12**
Lysis Buffer	3 x 10 ml
Disruption Buffer	35 ml
Mitochondria Storage Buffer	100 ml
Mitochondria Purification Buffer	20 ml
Protease Inhibitor Solution (100x)	2 x 300 µl

Storage

Lysis Buffer should be stored at –20°C upon arrival. All other buffers and Protease Inhibitor Solution (100x) should be stored at 2–8°C.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, Qproteome Kits are tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany, Tel: +49-6131-19240

Product Use Limitations

The Qproteome Mitochondria Isolation Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

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QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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Introduction

Proteomic analysis of organelles or specific groups of proteins is a powerful strategy for the discovery of proteins that are involved in specific cellular functions or disease. Targeted enrichment of specific groups of proteins or subcellular organelles reduces the complexity of samples and simplifies such approaches.

As they are the organelles in animal and plant cells in which oxidative phosphorylation takes place, mitochondria are the site of energy production in eukaryotic cells. In tissues that require large amounts of energy (e.g., skeletal muscle, kidney, or the pancreas), there are noticeably more mitochondria than in tissues with lower energy requirements. Mitochondria are compartmentalized by an outer and highly folded inner membrane. The inner membrane encloses the mitochondrial matrix, which contains the enzymes that take part in the Krebs respiratory cycle. The molecules that perform electron transfer and ATP synthesis are located on folds (cristae) on the inner surface of the inner membrane.

Due to their fundamental importance in biochemistry, mitochondria are a subject of intense research. Mitochondrial dysfunction can lead to serious and debilitating medical conditions, such as cancer, infertility, diabetes, heart diseases, blindness, deafness, kidney disease, liver disease, stroke, and migraine. Mitochondrial dysfunction is also involved in aging and neurodegenerative diseases such as Parkinson's and Alzheimer's disease.

Principle and Procedure

Washed cells or homogenized tissues are suspended in Lysis Buffer, which selectively disrupts the plasma membrane without solubilizing it, resulting in the isolation of cytosolic proteins. Plasma membranes and compartmentalized organelles, such as nuclei, mitochondria, and the endoplasmic reticulum (ER), remain intact and are pelleted by centrifugation. The resulting pellet is resuspended in Disruption Buffer, repeatedly passed through a narrow-gauge needle (to ensure complete cell disruption), and recentrifuged to pellet nuclei, cell debris, and unbroken cells. The supernatant (which contains mitochondria and the microsomal fraction) is recentrifuged to pellet mitochondria. After removal of the supernatant, mitochondria are washed and resuspended in Mitochondria Storage Buffer.

For high-purity preparations, the mitochondria pellet is resuspended in Mitochondria Purification Buffer and carefully pipetted on top of layers of Purification Buffer and Disruption Buffer. During a subsequent separation, mitochondria migrate through the liquid to form a band towards the bottom of the tube. The band is removed and the high-purity mitochondria are pelleted in Mitochondria Storage Buffer.

The procedure has been used successfully with several different mammalian cell lines, including HeLa, Cos7, NIH, and HEK293, and a number of different tissues, including liver, heart, and brain. Depending on the cell line, the yield from a single fractionation procedure is 20–80 µg protein from 5 x 10^6 cells. For some downstream applications, concentration of fractions may be necessary. A protocol for protein concentration using acetone precipitation can be found on page 12.

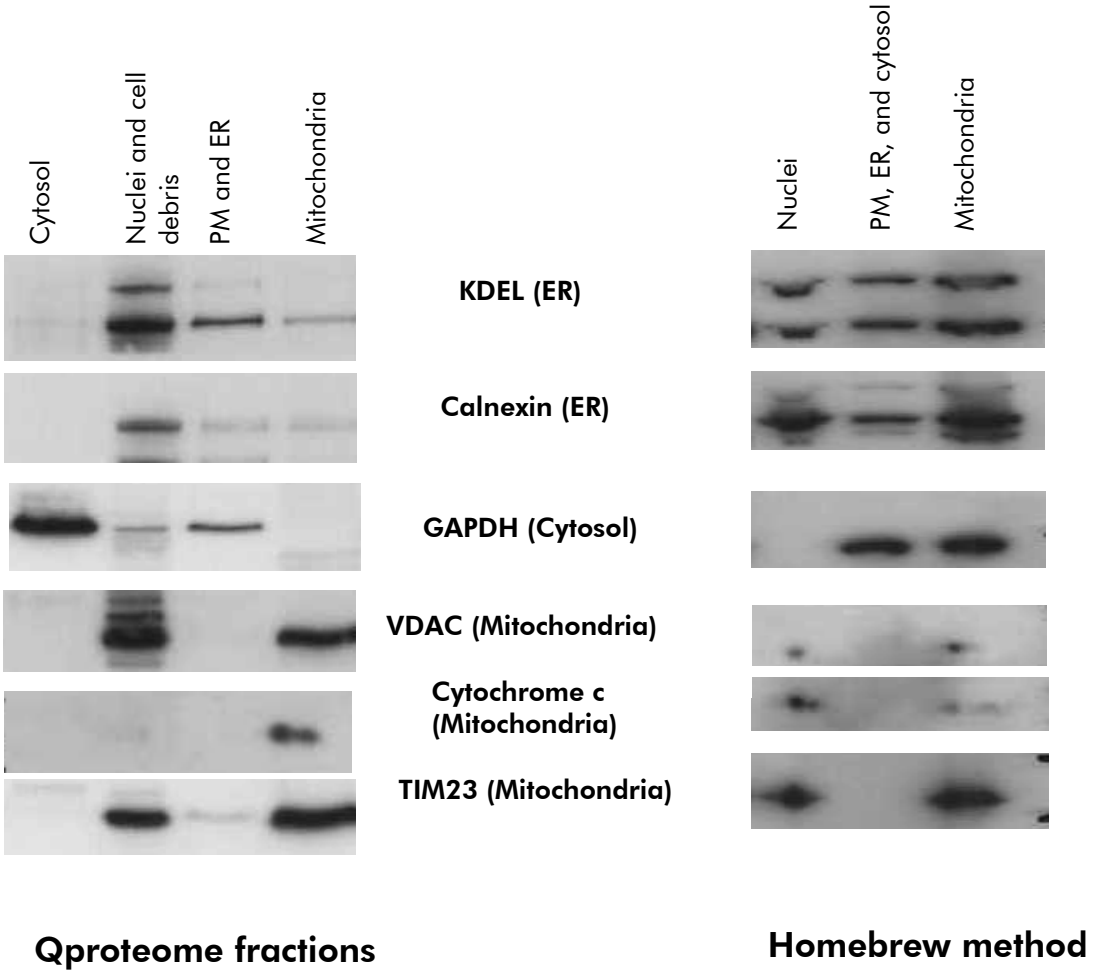
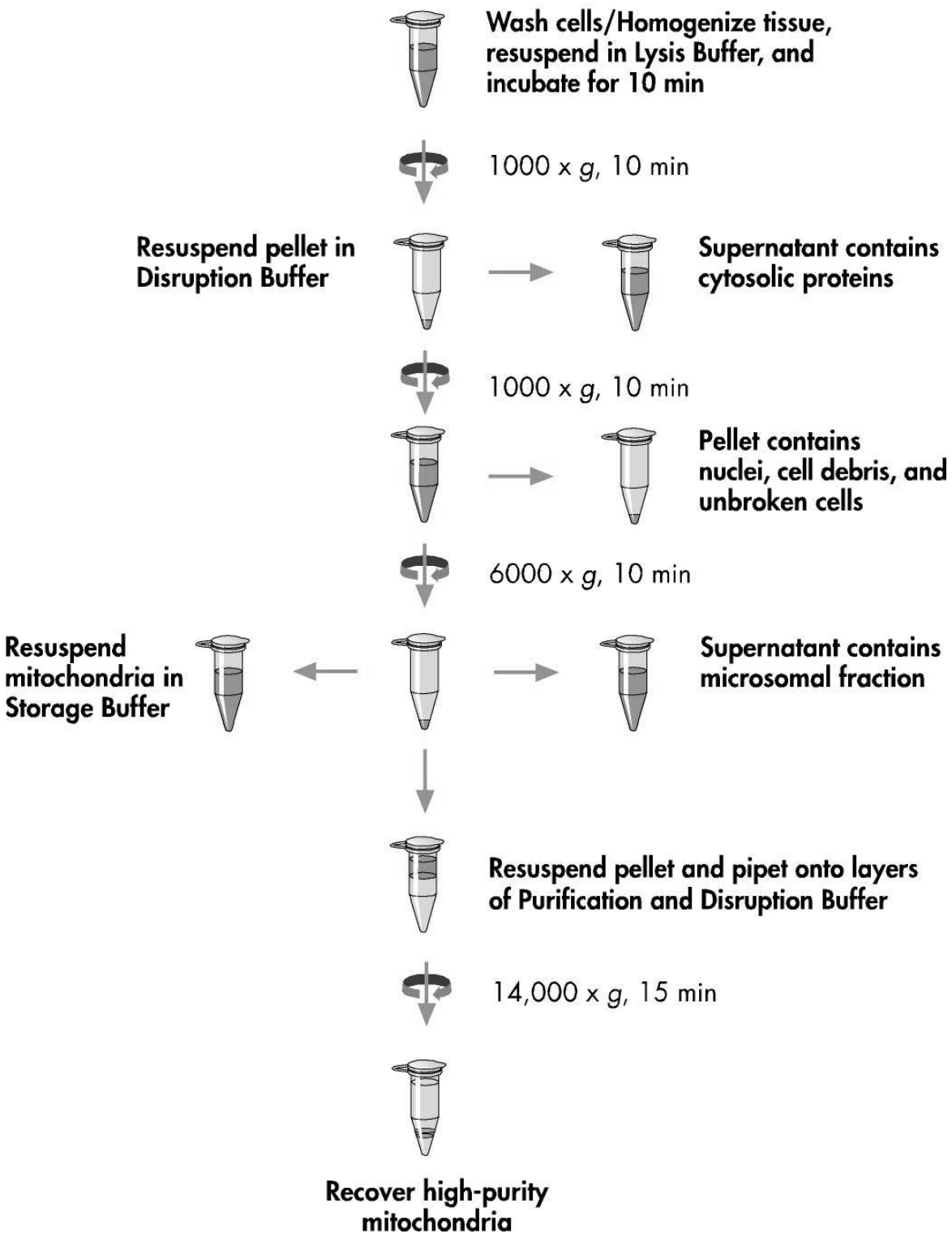


Figure 1 Qproteome kit delivers cleaner mitochondrial preparations than a "homebrew" method. Mitochondria were prepared from COS cells using either the Qproteome Mitochondria Isolation Kit or a "homebrew" method based on differential centrifugation. 20 µg protein from each fraction was separated on a 12% SDS-PAGE gel. Western blots were then probed with antibodies specific for cytosol-/organelle-/cell-compartment specific marker proteins. **PM**: plasma membrane; **ER**: endoplasmic reticulum.

Qproteome Mitochondria Isolation Procedure



Protocol: Isolation of Mitochondria from Eukaryotic Cell Lysates

This protocol is suitable for processing 5 x 10^6 to 2 x 10^7 cells.

Equipment and reagents be supplied by the user

- 0.9% (w/v) sodium chloride solution*
- End-over-end shaker
- Blunt-ended needle (26 or 21 gauge) and syringe (1 ml or 2 ml volume) for cell disruption and homogenization for example 26g (0.45 mm) x 23 mm
- Optional: Acetone stored at –20°C

Important notes before starting

- 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).

Things to do before starting

- 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]).

Procedure

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 pipette tip. Incubate for 10 min at 4°C on an end-over-end shaker.**

   Ensure that Protease Inhibitor Solution has been added to Lysis Buffer.
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.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

6. **Resuspend the cell pellet in 1.5 ml ice-cold Disruption Buffer by pipetting up and down using a 1 ml pipette 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.**

   Ensure that Disruption Buffer has been supplemented with Protease Inhibitor Solution.

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.

For standard preparations, proceed with step 10a. For high-purity mitochondria, proceed with step 10b.

Standard preparations

10a. Wash the mitochondrial pellet from step 9 with 1 ml Mitochondria Storage Buffer by carefully pipetting up and down using a 1 ml pipette tip. Centrifuge at 6000 x *g* for 20 min at 4°C.

11a. Resuspend the mitochondrial pellet in Mitochondria Storage Buffer or a buffer of choice for further analysis.

High-purity preparations

10b. Resuspend the pellet from step 9 in 750 µl Mitochondria Purification Buffer by carefully pipetting up and down using a 1 ml pipette tip. Pipet 750 µl Mitochondria Purification Buffer into a 2 ml microcentrifuge tube and slowly pipet 500 µl Disruption Buffer under the Mitochondria Purification Buffer. Carefully pipet the mitochondrial suspension on top of the Mitochondria Purification Buffer layer (see Figure 2).

Due to their different viscosities, Disruption Buffer and Mitochondria Purification Buffer do not readily mix, allowing them to be layered.

11b. Centrifuge at 14,000 x *g* for 15 min at 4°C.

A pellet or band containing mitochondria will form in the lower part of the tube.

12b. Carefully remove 1.5 ml of the supernatant without disturbing the mitochondria band or pellet.

The pellet is usually very soft. Take care when removing the supernatant that the pellet is not lost or disrupted. Save the supernatant for further analysis.

13b. Carefully remove the mitochondria band or pellet by aspirating the remaining 0.5 ml solution from the bottom of the tube and transfer to a new tube.

Note: It is important that the clear pellet (consisting of density gradient medium) that may be at the side of the tube is not disturbed. Due to its transparency, this pellet might be difficult to see.

14b. Dilute the suspension from step 13b with 1.5 ml Mitochondria Storage Buffer and centrifuge at 8000 x *g* for 10 min at 4°C.

15b. Remove 1.5 ml of the supernatant and dilute the remaining suspension with 1.5 ml Mitochondria Storage Buffer and centrifuge at 8000 x *g* for 10 min at 4°C.

16b. Repeat step 15b until the mitochondria form a pellet at the bottom of the tube.

Note: This procedure must be repeated until the mitochondria form a pellet at the bottom of the tube. The pellet is usually very soft. Take care when removing the supernatant that the pellet is not lost or disrupted.

17b. Resuspend the mitochondrial pellet in Mitochondria Storage Buffer or in a buffer of choice for further analysis.

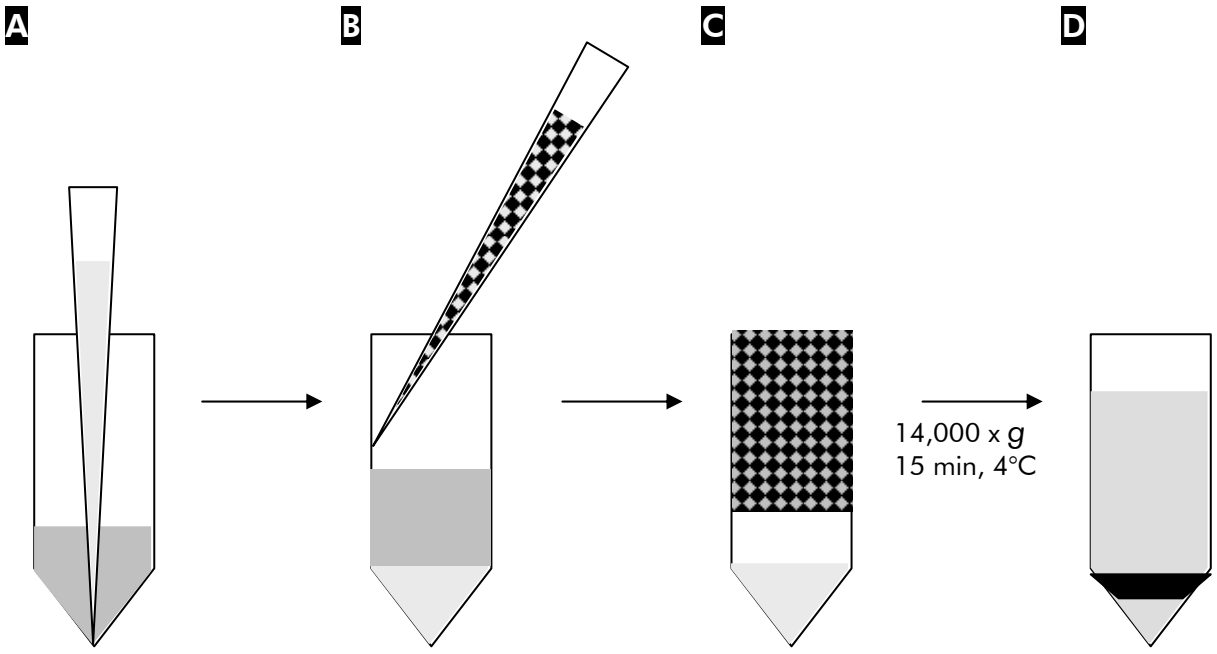


Figure 2 **A** In a 2 ml tube, 500 µl Disruption Buffer is pipetted under 750 µl Mitochondria Purification Buffer. **B** – **C** The crude mitochondria preparation from protocol step 9 is layered on top of the Mitochondria Purification Buffer. **D** After centrifugation, mitochondria are concentrated in a band towards the bottom of the tube.

Protocol: Isolation of Mitochondria from Tissues Using the Qproteome Mitochondria Isolation Kit

This protocol is suitable for processing tissues. In comprehensive tests using rat tissues, optimal sample sizes per preparation were found to be 60 mg (liver and heart) and 20 mg (brain).

Equipment and reagents be supplied by the user

- 2 ml reaction tubes
- 0.9% (w/v) sodium chloride solution*
- TissueRuptor (QIAGEN)
- End-over-end shaker
- Blunt-ended needle (26 or 21 gauge) and syringe (1 ml or 2 ml volume) for cell disruption and homogenization for example 26g x 23; 0.45 x 23
- Optional: Acetone stored at –20°C

Important notes before starting

- All steps are performed at 4°C. Use pre-cooled buffers. For long-term storage, separated protein fractions should be snap-frozen in liquid nitrogen and stored at –80°C.

Things to do before starting

- 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]).

Procedure

1. **Place freshly excised tissue on ice and remove an appropriately sized sample (see above). Wash the sample using 1 ml 0.9% (w/v) sodium chloride solution.**
2. **Cut sample into ~2 mm^3 pieces, place the pieces into a 2 ml reaction tube, and add 500 µl Lysis Buffer supplemented with Protease Inhibitor Solution.**

   Ensure that Protease Inhibitor Solution has been added to the Lysis Buffer.
3. **Homogenize the sample using the TissueRuptor rotor-stator homogenizer set at the lowest speed for 10s.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

4. **Pipet 1.5 ml Lysis Buffer supplemented with Protease Inhibitor Solution into the tube and incubate on an end-over-end shaker for 10 min at 4°C.**

   Ensure that Protease Inhibitor Solution has been added to the Lysis Buffer.

5. **Centrifuge the homogenate at 1000 x *g* for 10 min at 4°C.**
6. **Carefully remove the supernatant.**

   This fraction primarily contains cytosolic proteins.

7. **Resuspend the cell pellet in 1.5 ml ice-cold Disruption Buffer by pipetting up and down using a 1 ml pipette tip. Complete cell disruption by using a blunt-ended needle and a syringe (not provided). Draw the lysate into the syringe and eject with one stroke. Repeat 10 times. Alternatively, disrupt cells using a Dounce or Potter homogenizer.**

   Ensure that Disruption Buffer has been supplemented with Protease Inhibitor Solution.

8. **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 7 and 8 using 500 µl ice-cold Disruption Buffer. The supernatants from each extraction should be combined before the next step.

9. **Centrifuge the supernatant(s) from step 8 at 6000 x *g* for 10 min at 4°C.**
10. **Carefully remove the supernatant.**

    The pellet contains mitochondria. The supernatant constitutes the microsomal fraction.

For standard preparations, proceed with step 11a. For high-purity mitochondria, proceed with step 11b.

Standard preparations

11a. Wash the mitochondrial pellet from step 10 with 1 ml Mitochondria Storage Buffer by carefully pipetting up and down using a 1 ml pipette tip. Centrifuge at 6000 x *g* for 20 min at 4°C.

12a. Resuspend the mitochondrial pellet in Mitochondria Storage Buffer or a buffer of choice for further analysis.

High-purity preparations

11b. Resuspend the pellet from step 10 in 750 µl Mitochondria Purification Buffer by carefully pipetting up and down using a 1 ml pipette tip. Pipet 750 µl Mitochondria Purification Buffer into a 2 ml microcentrifuge tube and slowly pipet 500 µl Disruption Buffer under the Mitochondria Purification Buffer. Carefully pipet the mitochondrial suspension on top of the Mitochondria Purification Buffer layer (see Figure 3).

Due to their different viscosities, Disruption Buffer and Mitochondria Purification Buffer do not readily mix, allowing them to be layered.

12b. Centrifuge at 14,000 x *g* for 15 min at 4°C.

A pellet or band containing mitochondria will form in the lower part of the tube.

13b. Carefully remove 1.5 ml of the supernatant without disturbing the mitochondria band or pellet.

The pellet is usually very soft. Take care when removing the supernatant that the pellet is not lost or disrupted. Save the supernatant for further analysis.

14b. Carefully remove the mitochondria band or pellet by aspirating the remaining 0.5 ml solution from the bottom of the tube and transfer to a new tube.

Note: It is important that the clear pellet (consisting of density gradient medium) that may be at the side of the tube is not disturbed. Due to its transparency, this pellet might be difficult to see.

15b. Dilute the suspension from step 14b with 1.5 ml Mitochondria Storage Buffer and centrifuge at 8000 x *g* for 10 min at 4°C.

16b. Remove 1.5 ml of the supernatant and dilute the remaining suspension with 1.5 ml Mitochondria Storage Buffer and centrifuge at 8000 x *g* for 10 min at 4°C.

17b. Repeat step 16b until the mitochondria form a pellet at the bottom of the tube.

Note: This procedure must be repeated until the mitochondria form a pellet at the bottom of the tube. The pellet is usually very soft. Take care when removing the supernatant that the pellet is not lost or disrupted.

18b. Resuspend the mitochondrial pellet in Mitochondria Storage Buffer or in a buffer of choice for further analysis.

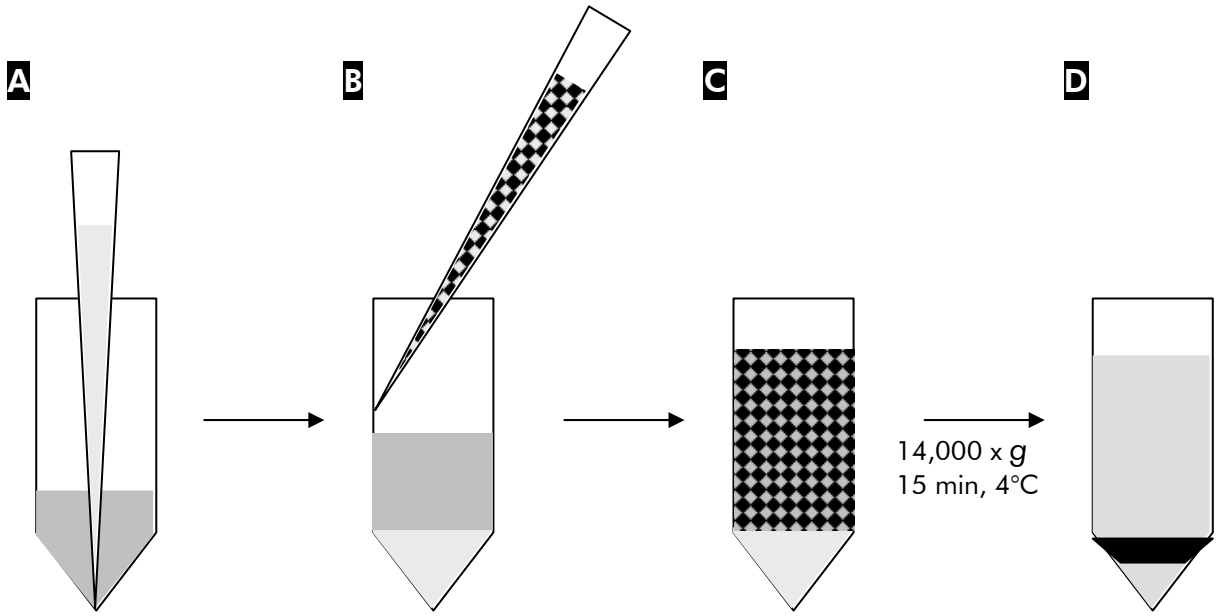


Figure 3 **A** In a 2 ml tube, 500 µl Disruption Buffer is pipetted under 750 µl Mitochondria Purification Buffer. **B** – **C** The crude mitochondria preparation from protocol step 10 is layered on top of the Mitochondria Purification Buffer. **D** After centrifugation, mitochondria are concentrated in a band towards the bottom of the tube.

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications such as 2D-PAGE.

1. **Add 4 volumes of ice-cold acetone to the protein fraction and incubate for 15 min on ice.**
2. **Centrifuge for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Discard the supernatant and wash the pellet with 1 ml 80% acetone.**
3. **Air dry the pellet.**

   Do not overdry the pellet as this may make it difficult to resuspend.

Troubleshooting Guide

	Comments and Suggestions
Inconsistent results in protein quantification assays	
Buffers contain components that may interfere with protein quantification assays	Use a protein assay that includes a precipitation step to remove interfering substances. Alternatively precipitate a portion of the eluate using acetone and dissolve the protein pellet in a reagent suitable for your protein assay.
Low mitochondria yield despite sufficient cells in starting material	
Cell disruption was not complete	In step 6 of the protocol, increase the number of lysate passages through the syringe needle. Instead of a needle and syringe, use a Potter or Dounce homogenizer to disrupt the cells.

Ordering Information

Product	Contents	Cat. no.
Qproteome Mitochondria Isolation Kit	Buffers and reagents for 12 high-purity mitochondrial preparations	37612
Related products		
Qprotome Mammalian Protein Prep Kit	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase® Nuclease	37901
Qproteome Plasma Membrane Protein Kit	Buffers and reagents for 6 high-purity plasma membrane protein preparations	37601
Qproteome Nuclear Protein Kit	For 6 nuclear protein preparations: Buffers, Reagents, Protease Inhibitor Solution, Benzonase® Nuclease	37582
Qproteome Nuclear Subfractionation Kit	For 6 nuclear protein preparations: Buffers, Reagents, Nuclear protein Fractionation Columns (6), Nuclear Protein Fractionation Resin, Protease Inhibitor Solution, Benzonase® Nuclease	37531
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
Qproteome Murine Albumin Depletion Kit	For albumin depletion of 6 murine serum or plasma samples: Murine Albumin Depletion Spin Columns (6)	37591
Qproteome Total Glycoprotein Kit	For 6 total glycoprotein preps: Buffers, Lectin Spin Columns (6), Detergent Solution, Protease Inhibitor Solution, Collection Tubes (6 x 2 ml)	37541
Qproteome Mannose Glycoprotein Kit	For 6 mannose glycoprotein preps: ConA, GNA, and LCH Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37551

Product	Contents	Cat. no.
Qproteome Sialic Glycoprotein Kit	For 6 sialic acid glycoprotein preps: WGA, SNA, and MAL Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37561
Qproteome O-Glycan Glycoprotein Kit	For 6 O-glycan glycoprotein preps: AIL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase® Nuclease	37512
Qproteome Cell Compartment Kit	For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase® Nuclease	37502
PhosphoProtein Purification Kit (6)	6 PhosphoProtein Purification Columns, 6 Nanosep® Ultrafiltration Columns, Reagents, Buffers	37101
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	9001271* 9001272† 9001273‡ 9001274§
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890

* 120 V, 60 Hz (for North America and Japan); † 235 V, 50/60 Hz (for Europe, excluding UK and Ireland); ‡ 235 V, 50/60 Hz (for UK and Ireland); § 235 V, 50/60 Hz (for Australia)

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Notes

Notes

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Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

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Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

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Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

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Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

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