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Qproteome[®] Mammalian Protein Preparation Handbook

For preparation of total protein from
mammalian cells



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Kit Contents

Qproteome Mammalian Protein Prep Kit	
Catalog no.	37901
Number of preps	100
Mammalian Cell Lysis Buffer	100 ml
Benzonase® Nuclease	2000 Units (25 U/μl)
Protease Inhibitor Solution (100x)	4 x 300 μl
Handbook	1

Storage

All kit components should be stored at 2–8°C. For longer storage, Benzonase® Nuclease should be stored at –20°C.

Product Use Limitations

The Qproteome Mammalian Protein Prep 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.

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 inside back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Qproteome Kits or QIAGEN products in general, please do not hesitate to contact us.

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.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside back cover).

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of Qproteome Kits is 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

Introduction

In order to be able to efficiently purify and analyze proteins, they must first be released from their host cell in a soluble form. Therefore, the first step in protein purification and analysis is cell lysis. Mammalian cells are bounded by a plasma membrane, which is composed of phospholipids and proteins. In contrast to bacterial or yeast cells, this membrane can be easily disrupted, but any lysis method should be gentle enough to deliver active, native-conformation proteins.

The Qproteome Mammalian Protein Prep Kit provides gentle but efficient detergent-based lysis of mammalian cells to deliver a total protein fraction suitable for any downstream application. Cell pellets are incubated with Mammalian Cell Lysis Buffer that is supplemented with Benzonase[®] Nuclease — an enzyme that digests nucleic acids — and a protease inhibitor, which protect the released proteins from enzymatic degradation. No specialized equipment is required. Cells are simply incubated with lysis buffer and centrifuged to separate cell debris. The supernatant contains the total protein fraction, which can be further purified or directly analyzed, for example by SDS-PAGE.

Table 1. Protein Yields and Protein Solubilization Using the Qproteome Mammalian Protein Prep Kit

Cell line	Protein yield (μg)*	Solubilization (%)*
Cos-7	900	93
HeLa	1700	97
CHO	800	98
NIH	940	97
Jurkat	780	96

* Average of 3 preparations, each of 5×10^6 cells.

Protocol: Isolation of Total Proteins from Mammalian Cells

The volumes given in this protocol are suitable for processing of $5\text{--}10 \times 10^6$ mammalian cells. When processing larger or smaller cultures, adjust the volume of buffer used accordingly.

Equipment and reagents be supplied by the user

- Ice-cold PBS
- Cell scraper
- 15 ml conical tube
- Microcentrifuge tubes
- Microcentrifuge
- Rotary shaker

Important notes before starting

- It is possible that the Protease Inhibitor does not thaw at room temperature. If this is the case, heat the protease inhibitor solution to 37°C with agitation and cool to room temperature.
- When processing small volumes, it may be necessary to dilute Benzonase[®] Nuclease using Mammalian Lysis Buffer.

Procedure

Cell collection

1. Aspirate cell-culture medium from culture plate.
2. Wash cells twice with 5 ml ice-cold PBS.
3. Add 10 ml ice-cold PBS.
4. Remove cells from culture plate by gentle scraping with cell-scraper and transfer cells to a pre-chilled 15 ml conical tube.
5. Centrifuge cell suspension for 5 min at $450 \times g$ in a centrifuge pre-cooled to 4°C . Discard supernatant. Keep cell pellet on ice.

Cell lysis

6. Add 1 U Benzonase[®] Nuclease and $10 \mu\text{l}$ of Protease Inhibitor (100x) to 1 ml Mammalian Lysis Buffer.
7. Resuspend $5\text{--}10 \times 10^6$ cells in the Mammalian Lysis Buffer prepared in step 6 and incubate on a rotary shaker for 5 minutes at 4°C .

- 8. Centrifuge the suspension for 10 minutes at 14,000 x g in a microcentrifuge tube pre-cooled to 4°C.**
- 9. Transfer the supernatant into a new microcentrifuge tube.**

The supernatant contains the total protein fraction. For some applications, such as 2-D gel analysis, the protein fraction may need to be concentrated. This can be achieved by acetone precipitation (see below).

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications.

- 1. Add four 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 air dry the pellet.**

Do not overdry the pellet as this may make it difficult to resuspend.
- 3. Depending on the application, resuspend the pellet in the required sample buffer.**

For 2D-PAGE, an extra desalting step may be required.
- 4. Resuspend the pellet from step 2 in 100 µl 8M urea.**
- 5. Desalt the sample using a gel filtration device (e.g., Bio-Spin® 6, Bio-Rad cat. no. 732-6227).**
- 6. Repeat steps 1 to 3.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back page for contact information).

Comments and Suggestions

Inconsistent results in protein quantification assays

The extraction buffer contains components that may interfere with protein quantification assays

Perform a precipitation step (e.g., using acetone, see above) to remove interfering substances.

Ordering Information

Product	Contents	Cat. no.
Qprotome Mammalian Protein Prep Kit	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase [®] Nuclease	37901
Related products		
Qproteome Bacterial Protein Prep Kit	For soluble protein preparations from up to 4.5 liters of bacterial culture: Lysis Buffer, Lysozyme, Benzonase [®] Nuclease	37900
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: ALL 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
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase [®] Nuclease	37512
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

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