July 2005

# Qproteome™ Nuclear Protein Handbook

For isolation of nuclear and nucleic-acid binding proteins from eukaryotic cell lysates

Contents

## Kit Contents

| **Qproteome Nuclear Protein Kit** | |
|---|---|
| **Catalog no.** | **37582** |
| **Number of preps** | **12** |
| Lysis Buffer NL | 2 x 10 ml |
| Extraction Buffer NX1 | 1 ml |
| Extraction Buffer NX2 | 2 x 1 ml |
| Detergent Solution NP | 2 x 0.25 ml |
| DTT Stock Solution | 0.25 ml of a 1 M solution |
| Benzonase® | 2000 Units (25 U/µl) |
| Protease Inhibitor Solution (100x) | 300 µl |
| Handbook | 1 |

## Storage

Buffers NL, NX1, and NX2 should be stored at room temperature (15–25°C).

Detergent Solution NP and Protease Inhibitor Solution (100x) should be stored at 4°C.

Benzonase® and DTT Stock Solution should be stored at –20°C.

## Product Use Limitations

Qproteome Nuclear Protein Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

## Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of QIAamp MinElute Media Kit is tested against predetermined specifications to ensure consistent product quality.

## 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 Nuclear Protein 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).

## 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

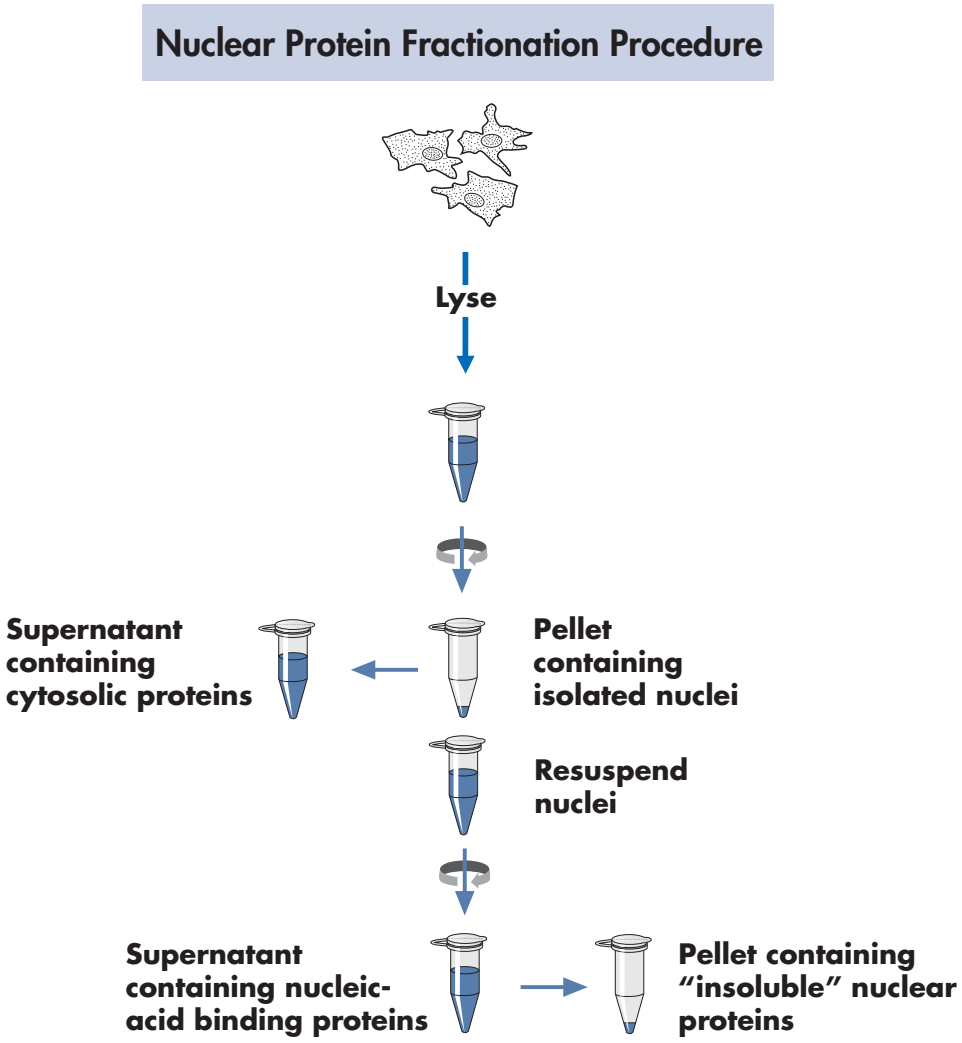
# Introduction

The functional architecture of the eukaryotic cell nucleus is of great interest to cell biologists. The identification of nuclear proteins — especially nucleic-acid–binding proteins (e.g., transcription factors) — is important for an understanding of genome regulation and function, and provides clues about the molecular function of novel proteins.

The nucleus contains a cell's genetic information and is the site of gene expression. Biological processes involving nucleic acids, such as transcription, replication, recombination, and DNA repair all involve the action of proteins that bind nucleic acids in a sequence-specific manner. These proteins interact with other proteins, which may or may not bind directly to nucleic acids. As a result of these interactions, large functional complexes are formed and anchored to specific nucleic acid sites. Many auxiliary proteins, enzymes, and complexes in the nucleus have important, general functions and are present in small to moderate amounts. However, proteins that bind at selected sites, such as transcription factors bound to specific promoters, represent only 0.01–0.001% of total cellular protein.

**Fractionation of Cell Lysate**


**Figure 1** Fractions obtained after processing cell lysates using the Qproteome Nuclear Protein Kit. **C**: Cytosolic fraction; **D**: nucleic-acid–binding protein fraction; **I**: "Insoluble" fraction.




## Principle and procedure

The Qproteome Nuclear Protein Kit is designed for specific enrichment of nuclear proteins. The kit provides standardized sample preparation for reliable quantitative and qualitative analysis of proteins for targeted proteomics. Nuclear proteins are separated from complex protein mixtures, allowing detection of low-abundance proteins (e.g., most transcription factors). Starting material for one fractionation procedure is 5 x $10^6$–1 x $10^7$ cells. The procedure has been used successfully with several different mammalian cell lines including HeLa, Jurkat, NIH3T3, HEK293, and Cos (Table 1, page 8). QIAGEN also offers the Qproteome Nuclear Subfractionation Kit (cat. no. 37531) which provides further subfractionation of the nucleic acid binding protein fraction.

**Table 1. Typical Total Protein Yields in Cytosolic, DNA Binding Protein, and Histone Fractions**

| Cell type | Number of cells processed | Cytosolic fraction | Nucleic-acid–binding proteins | Histones |
|---|---|---|---|---|
| HeLa | $5.4 \times 10^6$ | 1.9 mg | 0.34 mg | 0.40 mg |
| HEK293 | $4.0 \times 10^7$ | 9.4 mg | 0.55 mg | 0.89 mg |
| Jurkat | $1.5 \times 10^7$ | 2.6 mg | 0.23 mg | 0.53 mg |
| Cos | $3.7 \times 10^6$ | 2.9 mg | 0.14 mg | 0.18 mg |
| NIH3T3 | $7.9 \times 10^6$ | 1.9 mg | 0.28 mg | 0.34 mg |

### Preparation of the cytosolic fraction

Cells are incubated in hypotonic buffer, causing them to swell. Detergent added to the lysis buffer ruptures the plasma membrane and centrifugation is used to separate the cytosolic fraction (supernatant) from the cell nuclei (pellet).

### Preparation of the nucleic-acid binding protein fraction

The nuclear pellet is washed to remove cytosolic contaminants from the isolated cell nuclei. Washed cell nuclei are incubated in a buffer containing a high concentration of salt. During this incubation step, the nuclei shrink and nucleic-acid–binding proteins (e.g., transcription factors) separate from nucleic acids and diffuse through the nuclear pore to the exterior of the nucleus. A centrifugation step separates the nucleic-acid–binding proteins (which are found in the supernatant) from the nuclear debris, which includes genomic DNA (the pellet). The expected yield from one fractionation procedure is 200–300 µg specific nucleic-acid–binding proteins, at a concentration of approximately 5 µg/µl. This nucleic-acid–binding protein fraction can be used for activity tests (e.g., gel-shift assays, transcription factor activity assays) and characterization of function without further processing.

### Preparation of the “insoluble” nuclear protein fraction

Extraction of “insoluble” nuclear proteins is performed by incubation of the nuclear debris with a buffer containing Benzonase®, a DNase/RNase which digests genomic DNA and releases nuclear proteins intimately associated with DNA (e.g., histones).

# Protocol: Isolation of Nuclear Proteins from Mammalian-Cell Lysates

### Equipment and reagents to be supplied by the user

- Ice-cold PBS
- Cell scraper
- 15 ml conical tube
- Microcentrifuge tubes
- Thermomixer or similar
- Optional: Acetone stored at –20°C

### Important notes before starting

- All steps are performed at 4°C. Use pre-cooled buffers and equipment. Separated protein fractions should be stored at –80°C.
- For downstream applications, such as 2-D gel analysis, fractions may need to be concentrated. This can be achieved by acetone precipitation (see page 11).
- Starting material for one fractionation procedure using the Nuclear Protein Kit protocol is 5 x $10^6$ – 1 x $10^7$ cells.

### Things to do before starting

- Dilute 10 µl of the 1 M DTT Stock solution with 90 µl deionized, sterile water to give a concentration of 0.1 M. Store 0.1 M DTT Solution at –20°C.
- Immediately before starting the protocol, supplement the buffers used in the protocol with Protease Inhibitor Solution, DTT Stock Solution, and Benzonase® as shown in Table 2.

**Table 2. Preparing Buffers for the Qproteome Nuclear Protein Kit Protocol**

| Buffer | Required volume per prep. | Protease Inhibitor Solution | 0.1 M DTT | Benzonase® |
|---|---|---|---|---|
| Lysis Buffer NL (for protocol steps 6 and 11) | 1000 µl | 10 µl | 5 µl | – |
| Extraction Buffer NX1 (step 14) | 50 µl | 0.5 µl | – | – |
| Extraction Buffer NX2 (step 17) | 100 µl | 1 µl | 1 µl | 1 µl |

## 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 x *g* in a centrifuge pre-cooled to 4°C. Discard supernatant. Keep cell pellet on ice.

### Cell lysis and isolation of cell nuclei

6. Gently resuspend the cells collected in step 5 in 500 µl Lysis Buffer NL (supplemented with Protease Inhibitor Solution and 0.1 M DTT, see Table 2) by pipetting up and down several times. Incubate for 15 min on ice.
7. Transfer the resuspended cells to a clean pre-chilled microcentrifuge tube.
8. Add 25 µl Detergent Solution NP to the cell suspension and vortex for 10 s at maximum speed.
9. Centrifuge cell suspension for 5 min at 10,000 x *g* in a microcentrifuge pre-cooled to 4°C.
10. Transfer the supernatant (cytosolic fraction) into a new microcentrifuge tube and store at –80°C.
11. Resuspend the pellet (which contains cell nuclei) in 500 µl Nuclear Protein Lysis Buffer NL (supplemented with Protease Inhibitor Solution and 0.1 M DTT, see Table 2) by vortexing for 5 s at maximum speed.
12. Centrifuge the suspension of nuclei for 5 min at 10,000 x *g* in a pre-cooled microcentrifuge at 4°C.
13. Discard the supernatant and save the nuclear pellet.

### Extraction of nucleic-acid–binding proteins

14. Resuspend the nuclear pellet from step 13 in 50 µl Extraction Buffer NX1 (supplemented with Protease Inhibitor Solution, see Table 2) by pipetting up and down. Incubate suspension for 30 min with gentle agitation (e.g., 750 rpm in a Thermomixer) at 4°C.
15. Centrifuge the suspension of nuclei for 10 min at 12,000 x *g* in a microcentrifuge pre-cooled to 4°C.

16. **Transfer the supernatant (which contains nucleic-acid binding proteins) into a new microcentrifuge tube.**

    Store the supernatant and the pellet at –80°C until you are ready to proceed with further analysis. This fraction can be used directly for activity assays (e.g., gel-shift assays).
    Continue with step 17 to use the pellet for extraction of "insoluble" nuclear proteins (e.g., histones).

**Extraction of "insoluble" nuclear proteins (e.g., histones)**

17. **Resuspend the pellet from step 16 in 100 µl Extraction Buffer NX2 (supplemented with Benzonase®, Protease Inhibitor Solution, and 0.1 M DTT Stock Solution, see Table 2) by pipetting up and down. Incubate suspension for 1 h with gentle agitation (e.g., 750 rpm in a Thermomixer) at 4°C.**
18. **Centrifuge pellet suspension for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Transfer the supernatant to a new microcentrifuge tube and store at –80°C.**

## Protocol: Acetone Precipitation of Protein Fractions

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

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.**

# 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 |
|---|---|
| **Low protein concentration in protein fractions** | |
| Too few cells in starting material | Use a minimum of 5 x $10^6$ cells per preparation (see Table 1). |
| **Poor protein compartmentalization** | |
| a) Incomplete removal of cytosolic fraction | Ensure that all traces of supernatant are removed after the nuclear pellet wash step. |
| b) Nuclei are disrupted during nucleic-acid–binding protein extraction | Gently resuspend the nuclear pellet in extraction buffer NX1 by pipetting up and down. Do not vortex. |
| **No or low protein activity** | |
| Fractionated proteins are degraded | Ensure all buffers and equipment are cooled to 4°C during the entire procedure. Ensure that Protease Inhibitor Solution has been added to buffers. Snap-freeze eluted proteins using liquid nitrogen. |

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Qproteome Nuclear Protein Kit | For 12 nuclear protein preparations: Buffers, Reagents, Protease Inhibitor Solution, Benzonase® | 37582 |
| **Related products** | | |
| Qproteome Nuclear Subfractionation Kit | For 6 nuclear protein preparations: Buffers, Reagents, Nuclear protein Fractionation Columns (6), Nuclear Protein Fractionation Resin, Protease Inhibitor Solution, Benzonase® | 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 Bacterial Protein Prep Kit | For soluble protein preparations from up to 4.5 liters of bacterial cell culture: Lysis Buffer, Lysozyme, Benzonase® | 37900 |
| Qprotome Mammalian Protein Prep Kit | For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase® | 37901 |
| 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. |
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| 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® | 37512 |
| Qproteome Cell Compartment Kit | For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase® | 37502 |

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