Second Edition June 2011

# Qproteome® Nuclear Protein Handbook

Qproteome Nuclear Protein Kit

For isolation of nuclear and nucleic acid binding proteins from eukaryotic cells and tissues

QIAGEN®

Sample & Assay Technologies

# QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

**QIAGEN sets standards in**:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

# Contents

## Kit Contents

| **Qproteome Nuclear Protein Kit** | |
|---|---|
| **Catalog no.**<br>**Number of preps** | **37582**<br>**12 (cell samples) or 10 (tissue samples)** |
| 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® Nuclease | 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® Nuclease and DTT Stock Solution should be stored at –20°C.

## Product Use Limitations

The Qproteome Nuclear Protein 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.

## Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of Qproteome Nuclear Protein Kits 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. 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 ).

## 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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Qproteome Glycoprotein 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 see our Technical Support center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com ).

## 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

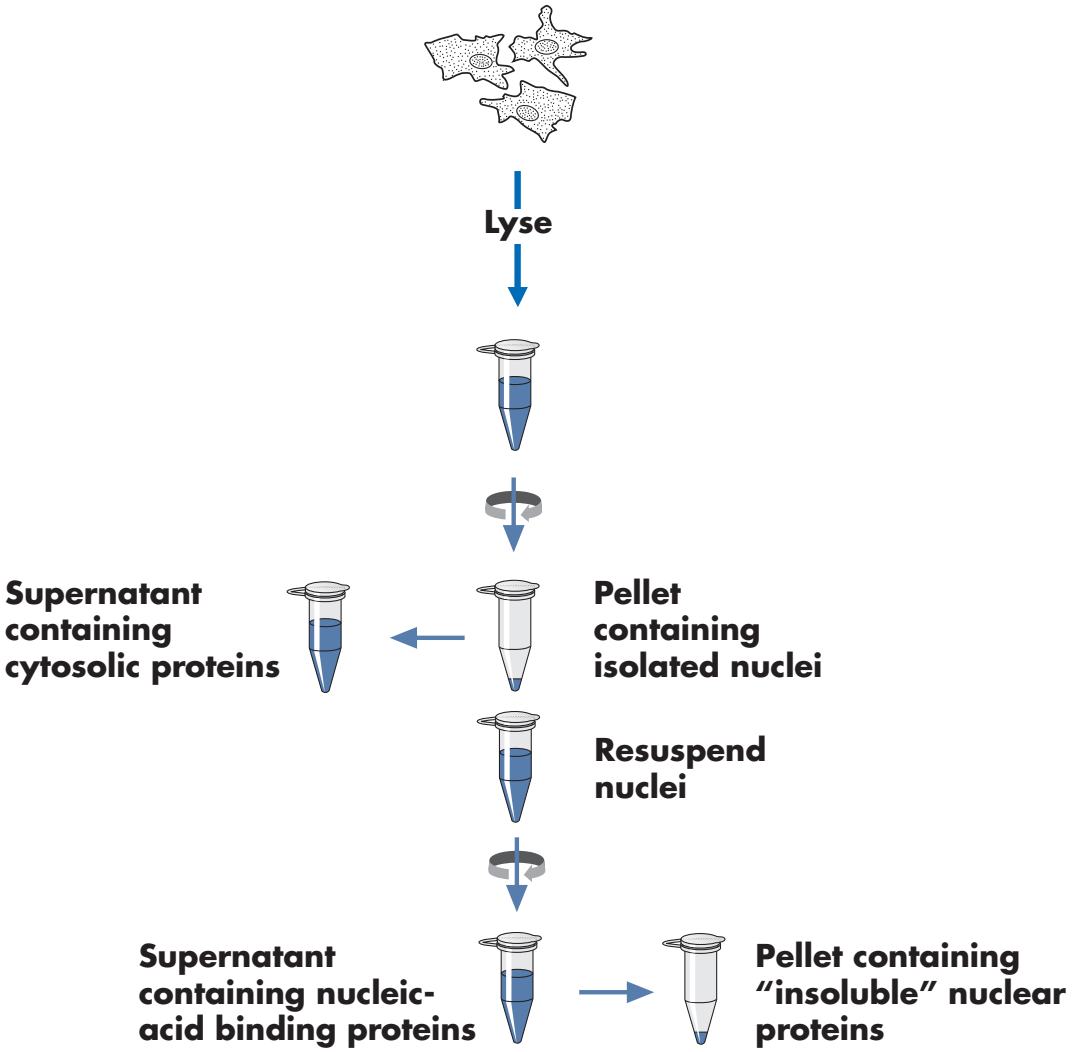
# Introduction

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.


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

## Nuclear Protein Fractionation Procedure



## 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 or 20 mg tissue. The cell protocol has been used successfully with several different mammalian cell lines including HeLa, Jurkat, NIH3T3, HEK293, and Cos (Table 1). The tissue protocol has been tested successfully with several rat tissues, including liver, heart, lung, and brain (Table 2).

**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 x $10^6$ | 1.9 mg | 0.34 mg | 0.40 mg |
| HEK293 | 4.0 x $10^7$ | 9.4 mg | 0.55 mg | 0.89 mg |
| Jurkat | 1.5 x $10^7$ | 2.6 mg | 0.23 mg | 0.53 mg |
| Cos | 3.7 x $10^6$ | 2.9 mg | 0.14 mg | 0.18 mg |
| NIH3T3 | 7.9 x $10^6$ | 1.9 mg | 0.28 mg | 0.34 mg |

**Table 2. Protein yields for rat tissue in cytosolic, DNA binding protein and histone Fractions**

| Tissue type | Cytosolic Fraction | DNA binding proteins | Histone |
|---|---|---|---|
| Brain | 1.2 mg | 71 µg | 45 µg |
| Liver | 3.4 mg | 83 µg | 222 µg |
| Heart | 1.6 mg | 145 µg | 540 µg |
| Lung | 1.5 mg | 76 µg | 296 µg |

### 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 for cell samples. 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® Nuclease, 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 Cells

### 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 14).
- 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® Nuclease as shown in Table 2.

**Table 2. Preparing buffers for the Qproteome Nuclear Protein Kit cell protocol**

| Buffer | Required volume per prep. | Protease Inhibitor Solution | 0.1 M DTT | Benzonase® Nuclease |
|---|---|---|---|---|
| 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® Nuclease, 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: Isolation of Nuclear Proteins from Mammalian Tissues

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

- Ice-cold PBS
- Homogenizer (e.g., TissueRuptor)
- 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 14).
- Starting material for one fractionation procedure using the Nuclear Protein Kit protocol is 20 mg tissue.

### 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® Nuclease as shown in Table 3.

**Table 3. Preparing buffers for the Qproteome Nuclear Protein Kit tissue protocol**

| Buffer | Required volume per prep. | Protease Inhibitor Solution | 0.1 M DTT | Benzonase® Nuclease |
|---|---|---|---|---|
| Lysis Buffer NL (for protocol steps 4 and 9) | 1500 µl | 15 µl | 7.5 µl | – |
| Extraction Buffer NX1 (step 12) | 100 µl | 1 µl | – | – |
| Extraction Buffer NX2 (step 15) | 100 µl | 1 µl | 1 µl | 1 µl |

## Procedure

### Lysis and isolation of cell nuclei

1. **Cut the tissue (20 mg) into 3–4 pieces. Wash with 1 ml ice-cold PBS.**
2. **Place tissue pieces into a 2 ml microcentrifuge tube and add 500 µl of Lysis Buffer NL (supplemented with Protease Inhibitor Solution and 0.1 M DTT, see Table 3).**
3. **Disrupt tissue for 10 s with a TissueRuptor set at the lowest speed.**
4. **Add 500 µl Lysis Buffer NL (supplemented with Protease Inhibitor Solution and 0.1 M DTT, see Table 3). Incubate for 15 min on ice.**
5. **Transfer the lysate to a clean pre-chilled microcentrifuge tube.**
6. **Add 50 µl Detergent Solution NP to the lysate and vortex for 10 s at maximum speed.**
7. **Centrifuge lysate for 5 min at 10,000 x *g* in a microcentrifuge pre-cooled to 4°C.**
8. **Transfer the supernatant (cytosolic fraction) into a new microcentrifuge tube and store at –80°C.**
9. **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 3) by vortexing for 5 s at maximum speed.**
10. **Centrifuge the suspension of nuclei for 5 min at 10,000 x *g* in a pre-cooled microcentrifuge at 4°C.**
11. **Discard the supernatant and save the nuclear pellet.**

### Extraction of nucleic-acid–binding proteins

12. **Resuspend the nuclear pellet from step 11 in 100 µl Extraction Buffer NX1 (supplemented with Protease Inhibitor Solution, see Table 3) by pipetting up and down. Incubate suspension for 30 min with gentle agitation (e.g., 750 rpm in a Thermomixer) at 4°C.**
13. **Centrifuge the suspension of nuclei for 10 min at 12,000 x *g* in a microcentrifuge pre-cooled to 4°C.**
14. **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 15 to use the pellet for extraction of "insoluble" nuclear proteins (e.g., histones).

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

15. **Resuspend the pellet from step 14 in 100 µl Extraction Buffer NX2 (supplemented with Benzonase® Nuclease, Protease Inhibitor Solution, and 0.1 M DTT Stock Solution, see Table 3) by pipetting up and down. Incubate suspension for 1 h with gentle agitation (e.g., 750 rpm in a Thermomixer) at 4°C.**
16. **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.

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com ).

| | 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 (cells) or 10 (tissues) nuclear protein preparations: Buffers, Reagents, Protease Inhibitor Solution, Benzonase® Nuclease | 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® Nuclease | 37531 |
| TissueRuptor | Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes | 9001271*<br>9001272†<br>9001273‡<br>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)

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

**www.qiagen.com**

**Australia** ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

**Austria** ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

**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

**Finland** ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

**France** ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

**Germany** ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

**Hong Kong** ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

**Ireland** ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

**Italy** ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

**Japan** ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

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

**Luxembourg** ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

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

**The Netherlands** ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

**Norway** ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

**Singapore** ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

**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

**USA** ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)


1068664 06/2011

Sample & Assay Technologies