

July 2005

Qproteome™ Bacterial Protein Preparation Handbook

For preparation of soluble proteins from
bacterial cell cultures



WWW.QIAGEN.COM

Trademarks: QIAGEN®, Qproteome™, (QIAGEN Group) Benzonase® (Merck KGaA, Germany), Coomassie® (ICI [Imperial Chemical Industries] Organics Inc.)

Benzonase endonuclease is covered by several patents, i.e. the US patent No. 5,173,418 and EP No. 0229866. Nycomed Pharma A/S, Denmark claims worldwide patent rights to Benzonase endonuclease which are licensed exclusively to MERCK KGaA, Darmstadt (Germany).

© 2005 QIAGEN, all rights reserved.

Contents

Kit Contents	4
Storage	4
Product Use Limitations	4
Product Warranty and Satisfaction Guarantee	4
Technical Assistance	5
Quality Control	5
Safety Information	5
Introduction	6
Protocol	7
■ Isolation of Soluble Protein Extracts from Bacterial Cells	7
Troubleshooting Guide	8
Ordering Information	9
QIAGEN Distributors	11

Kit Contents

Qproteome Bacterial Protein Prep Kit	
Catalog no.	37900
Native Lysis Buffer	3 x 60 ml
Benzonase [®]	3 x 80 µl (25 U/ml)
Lysozyme	3 x 60 mg
Handbook	1

Storage

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

Product Use Limitations

Qproteome 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.

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. Bacteria possess a rigid cell wall composed of peptidoglycans that must be disrupted to access cellular proteins. This has traditionally been accomplished by mechanical means, for example by subjecting cells to shear forces in a French press or by sonication. However, these methods are time-consuming and labor-intensive, and often lysis is incomplete.

The Qproteome Bacterial Protein Prep Kit provides gentle but efficient detergent-based lysis of bacterial cells to deliver a soluble protein fraction suitable for any downstream application. Cell pellets are incubated with Native Lysis Buffer that is supplemented with lysozyme and Benzonase[®], enzymes which help disrupt cell walls and digest nucleic acids respectively. No specialized equipment is required. Cells are simply incubated with lysis buffer and centrifuged to separate cell debris and insoluble protein (e.g., inclusion bodies). The supernatant contains the soluble protein fraction, which can be further purified or directly analyzed, for example by SDS-PAGE.

Protocol: Isolation of Soluble Protein Extracts from Bacterial Cells

The volumes given in this protocol are suitable for processing a cell pellet from a 250 ml bacterial culture. When processing larger or smaller cultures, adjust the volume of buffer used accordingly. This procedure exploits the disruption of bacterial cell walls by a freeze/thaw cycle. Therefore, before processing freshly harvested cell pellets, they must be frozen using liquid nitrogen or dry ice.

Things to do before starting

Before use, Native Lysis Buffer must be supplemented with lysozyme and Benzonase[®]. Dissolve the contents of a lysozyme vial in 600 μ l Native Lysis Buffer. Add 100 μ l of the lysozyme solution to each 10 ml of Native Lysis Buffer. Store the remaining lysozyme solution at -20°C . Thaw the vial containing Benzonase[®] solution and add 10 μ l to each 10 ml of Native Lysis Buffer.

Materials to be supplied by user

- Frozen cell pellet from 250 ml bacterial culture

Procedure

- 1. Thaw the cell pellet for 15 min on ice and resuspend the cells in 10 ml Native Lysis Buffer.**

Ensure that lysozyme and Benzonase[®] have been added to the lysis buffer.

- 2. Incubate on ice for 30 min.**
- 3. Mix 2–3 times by gently swirling the cell suspension.**
- 4. Centrifuge lysate at 14,000 x g for 30 min at 4°C to pellet the cellular debris. Retain the cell lysate supernatant.**

The supernatant contains the soluble fraction of the bacterial cell proteins.

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

Recombinant protein not found in soluble fraction

Protein forms inclusion bodies

The buffer in the Qproteome Bacterial Protein Prep Kit provides lysis under native conditions. Solubilization of inclusion bodies requires high concentrations of denaturants (e.g., 6M Guanidine HCl). Protocols for purification of proteins under denaturing conditions can be found in *The QIAexpressionist*, available in convenient PDF format at www.qiagen.com.

Ordering Information

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

QIAGEN Companies

Please see the back cover for contact information for your local QIAGEN office.

QIAGEN Distributors

Argentina

Tecnolab S.A.
Tel: (011) 4555 0010
Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Brazil

Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

Chile

Biosonda SA
Tel: 562 209 6770
Fax: 562 274 5462
E-mail: ventas@biosonda.cl
Web site: www.biosonda.cl

Croatia

INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail: inel-medicinska-tehnika@zg.htnet.hr

Cyprus

Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail: a.sarpetsas@biotronics.com.cy

Czech Republic

BIO-CONSULT spol. s.r.o.
Tel/Fax: (420) 2 417 29 792
E-mail: info@bioconsult.cz
Web site: www.bioconsult.cz

Egypt

Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Estonia

PEAL-Est OÜ
Tel: (051) 65 830
Fax: (07) 383 360
E-mail: langel@ut.ee

Greece

BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr
Web site: www.bioanalytica.gr

Hungary

Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

India

Genetix
Tel: (011)-2542 1714
or (011)-2515 9346
Fax: (011)-2546 7637
E-mail: genetix@nda.vsnl.net.in

Israel

Westburg (Israel) Ltd.
Tel: 08-6900655
or 1-800 20 22 20 (toll free)
Fax: 08-6900650
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea

LRS Laboratories, Inc.
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia

RESEARCH BIOLABS SDN. BHD.
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico

Quimica Valaner S.A. de C.V.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: ventas@valaner.com
Web site: www.valaner.com

New Zealand

Biolab Ltd
Tel: (09) 980 6700
or 0800 933 966
Fax: (09) 980 6788
E-mail: biosciences@nzl.biolabgroup.com
Web site: www.biolabgroup.com/nzl

Poland

Syngen Biotech Sp.z.o.o.
Tel: (071) 798 58 50 - 52
Fax: (071) 798 58 53
E-mail: info@syngen.pl
Web site: www.syngen.pl

Portugal

IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674
E-mail: consultasbiotec@izasa.es

Saudi Arabia

Abdulla Fouad Co. Ltd.
Tel: (03) 8324400
Fax: (03) 8346174
E-mail: sadiq.omar@abdulla-fouad.com

Singapore

Research Biolabs Pte Ltd
Tel: 6777 5366
Fax: 6778 5177
E-mail: sales@researchbiolabs.com
Web site: www.researchbiolabs.com

Slovak Republic

BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@cdicon.sk
Web site: www.bioconsult.cz

Slovenia

MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

South Africa

Southern Cross Biotechnology (Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za

Spain

IZASA, S.A.
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: consultasbiotec@izasa.es

Taiwan

TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand

Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

Turkey

Medek Medikal Ürünler ve Sağlık Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makialp@med-ek.com

All other countries

QIAGEN GmbH, Germany

Australia ■ **QIAGEN Pty Ltd** ■ PO Box 641 ■ DONCASTER VIC 3108

Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ **QIAGEN Vertriebs GmbH** ■ Löwengasse 47 / Top 6 ■ A-1030 Wien

Orders: 0800/28-10-10 ■ Fax: 0800/28-10-19 ■ Technical: 0800/28-10-11

Belgium ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands

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

Canada ■ **QIAGEN Inc.** ■ 2800 Argentia Road ■ Unit 7 ■ Mississauga ■ Ontario ■ L5N 8L2

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

China ■ **QIAGEN China Representative Office** ■ Room 2213 ■ POS PLAZA ■ Century Avenue 1600 ■

Pudong ■ Shanghai 200122 ■ Telephone 021-51162555 ■ Fax 021-51162500

France ■ **QIAGEN S.A.** ■ 3 avenue du Canada ■ LP 809 ■ 91974 COURTABOEUF CEDEX

Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ **QIAGEN GmbH** ■ QIAGEN Strasse 1 ■ 40724 Hilden

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

Italy ■ **QIAGEN S.p.A.** ■ Via Grosio, 10/10 ■ 20151 Milano

Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 02-33430414

Japan ■ **QIAGEN K.K.** ■ Forefront Tower II ■ 13-1, Kachidoki 3 Chome ■ Chuo-ku, Tokyo 104-0054

Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Luxembourg ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands

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

The Netherlands ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo

Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Switzerland ■ **QIAGEN AG** ■ Garstligweg 8 ■ 8634 Hombrechtikon

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

UK and Ireland ■ **QIAGEN Ltd.** ■ QIAGEN House ■ Fleming Way ■ Crawley ■ West Sussex, RH10 9NQ

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

USA ■ **QIAGEN Inc.** ■ 27220 Turnberry Lane ■ Suite 200 ■ Valencia ■ CA 91355

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

