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Qproteome[®] Bacterial Protein Preparation Handbook

For preparation of soluble proteins from
bacterial cell cultures



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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	
■ Isolation of Soluble Protein Extracts from Bacterial Cells	7
Troubleshooting Guide	8
Ordering Information	9

Kit Contents

Qproteome Bacterial Protein Prep Kit	
Catalog no.	37900
Native Lysis Buffer	3 x 60 ml
Benzonase [®] Nuclease	3 x 80 μ l (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[®] Nuclease should be stored at –20°C.

Product Use Limitations

The Qproteome Bacterial 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. 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[®] Nuclease, 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[®] Nuclease. 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[®] Nuclease 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[®] Nuclease 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 [®] Nuclease	37900
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 Nuclear Protein Kit	For 12 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 Mitochondria Isolation Kit	Buffers and reagents for 12 high-purity mitochondrial preparations	37612
Qproteome Plasma Membrane Protein Kit	Buffers and reagents for 6 high-purity plasma membrane protein preparations	37601

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