January 2005

Qproteome Soluble Protein Separation Handbook

For fractionation of protein samples used in proteomics analysis

Contents

Kit Contents

Qproteome Soluble Protein Separation Kit	
Catalog no.	**37512**
Number of preps	**10**
Lysis Buffer F	1 x 10 ml
Benzonase®	1 x 80 µl
Fractionation Buffer	1 x 15 ml
Precipitation Reagent FP1	1 x 250 µl
Precipitation Reagent FP2	1 x 2.5 ml
Protease Inhibitor Solution (100x)	1 x 300 µl

Storage

Fractionation Buffer and Precipitation Reagent FP2 should be stored at room temperature (15–25°C). Storage of Fractionation Buffer at a temperature lower than room temperature may lead to the formation of a precipitate. This precipitate can be dissolved by heating the buffer to 37°C with stirring. Any precipitate should be dissolved before use.

All other kit components should be stored at 2–8 °C.

For longer storage, Benzonase® can 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).

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.

The following risk and safety phrases apply to the components of the Soluble Protein Separation Kit:

Precipitation Reagent FP2

Contains trichloroacetic acid. Corrosive. Dangerous for the environment. Risk and safety phrases*: R35-50/53 S26-36/37/39-45-60-61

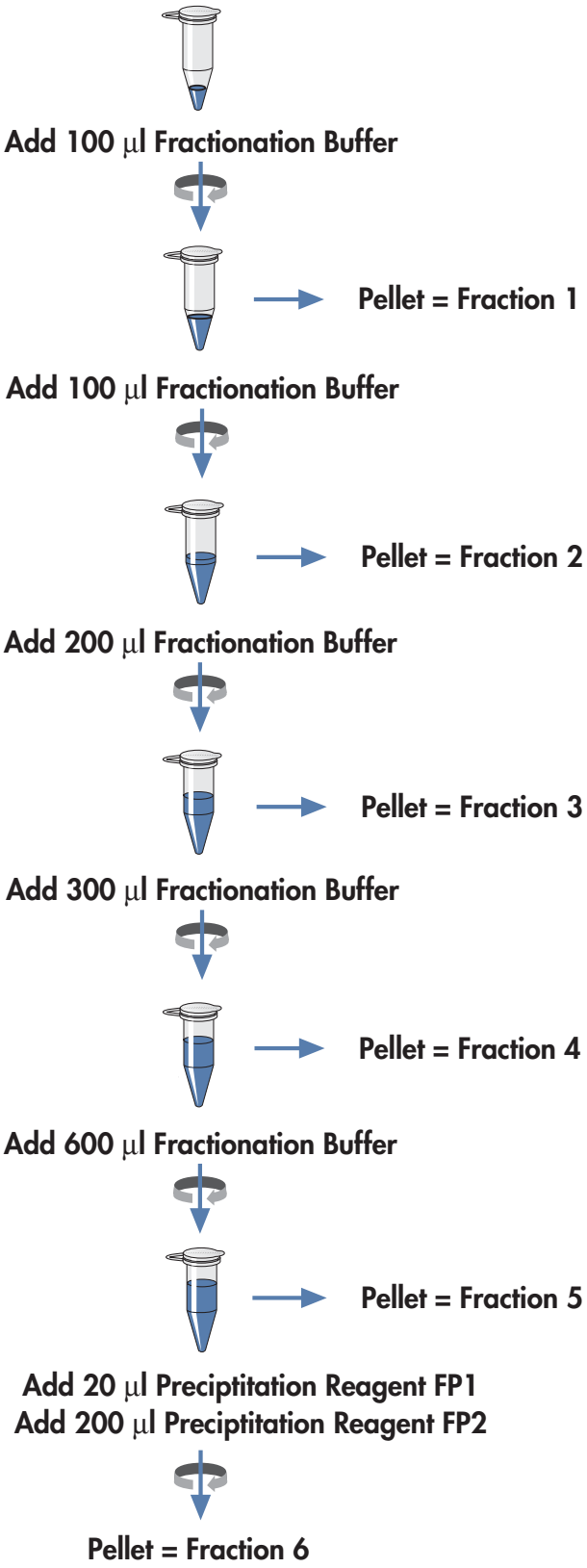
24-hour emergency information

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

* R35: Causes severe burns. R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37/39: Wear suitable protective clothing, gloves and eye/face protection. S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/Safety data sheets.

Soluble Protein Separation Procedure



Introduction

The sheer complexity of the proteome is the major problem facing proteomics researchers, with purification and quantification of low-abundance proteins being especially problematic. This problem can only be addressed through sample preparation or fractionation procedures. Such fractionation procedures can be based on different criteria and can vary in their complexity.

The Soluble Protein Separation Kit is designed for fast and easy fractionation of complex protein samples. Sequential addition of increasing amounts of Fractionation Buffer precipitates proteins that can be separated by centrifugation. Precipitated proteins can be redissolved in PBS or other appropriate buffer. The first five fractions will contain native proteins whereas the proteins in the last fraction will be denatured. However, the majority of proteins will precipitate in fractions 1 to 5.

Fractionation is useful prior to downstream applications, such as 2D-PAGE. For such applications, desalting all fractions using ultrafiltration, desalting columns, or acetone precipitation (see page 11) is recommended before analysis.

Fractionation of Proteins Using the Soluble Protein Separation Kit

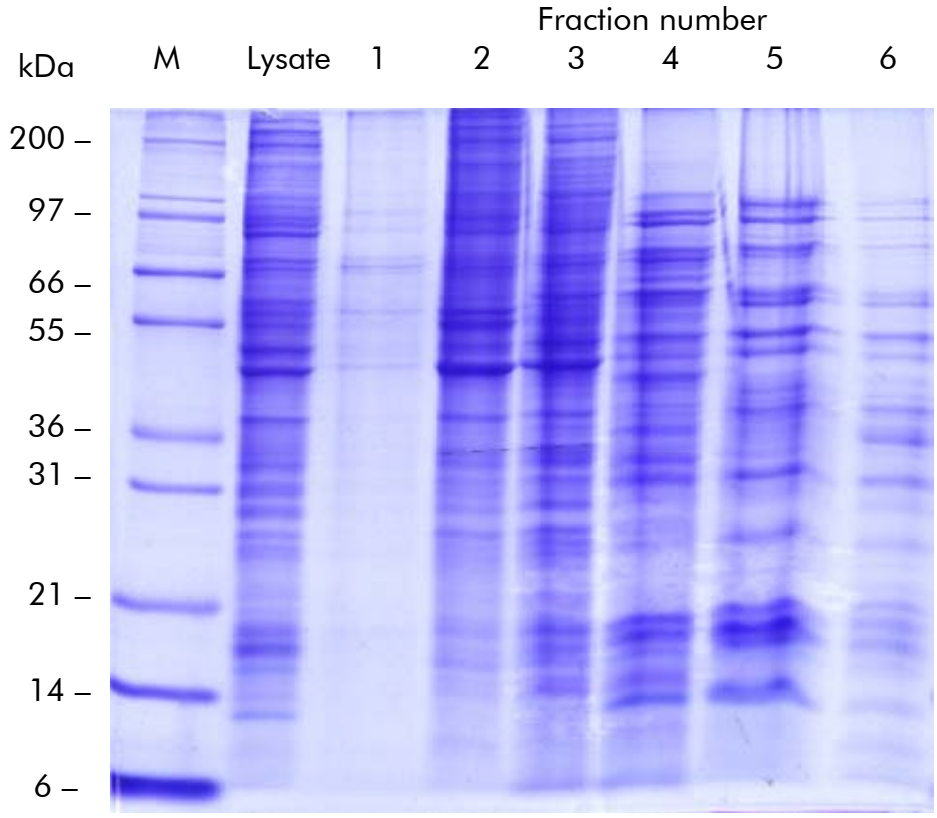


Figure 1 Fractionation of an NIH-3T3 cell lysate sample using the Soluble Protein Separation Kit. M: markers.

Preparation of Cleared Lysates from Mammalian Cells

To be supplied by user

- PBS (50 mM NaH_2PO_4 , 150 mM NaCl, pH 7.2)

Procedure

1. **Wash the cells with phosphate-buffered saline (PBS) and collect them by centrifugation for 5 min at 1000 x *g*.**
2. **Resuspend the cells in 500 µl Lysis Buffer F.**

   Optional: supplement Lysis Buffer F with 5 µl Protease Inhibitor Solution (100x) and 7 µl Benzonase® to prevent protein degradation and digest nucleic acids.
3. **Lyse the cells according to steps 3a or 3b.**

3a. **Lyse the cells by sonication on ice. Use six 15 s bursts at 75 W with a 10 s cooling period between each burst. Use a sonicator equipped with a microtip.**

3b. **Lyse cells by three consecutive freeze–thaw cycles with freezing on dry ice and thawing at room temperature (15–25°C).**

4. **Centrifuge the lysate at 10,000 x *g* for 10 min at 4°C to pellet cellular debris and DNA. Save the supernatant.**

   Note: The use of detergents might interfere with the fractionation procedure and is not recommended.

Soluble Protein Separation Kit Fractionation Protocol

Materials and reagents to be supplied by user

- Microcentrifuge tubes
- 1 x SDS-PAGE sample buffer (50 mM Tris·Cl, 10% glycerol, 1% SDS, 0.01% bromophenol blue, 50 mM DTT; pH 6.8)
- 80% (v/v) acetone, stored at –20°C
- Optional: 100% acetone stored at –20°C

Important notes before starting

- Fractionation Buffer contains components that may interfere with protein quantification assays. A precipitation step (e.g., using acetone, see page 11) to remove interfering substances should be performed before protein assay. Alternatively, the sample can be dialyzed against an appropriate buffer before determination of protein concentration.
- Storage of Fractionation Buffer at a temperature lower than room temperature may lead to the formation of a precipitate. This precipitate can be dissolved by heating the buffer to 37°C with stirring. Any precipitate should be dissolved before use.

Procedure

1. **Place 500 µl protein-containing sample (e.g., cleared cell lysate or plasma) into a 1.5 ml microcentrifuge tube and add 100 µl Fractionation Buffer. Vortex briefly and incubate on ice for 5 min.**
2. **Centrifuge at 14,000 x *g* at 4°C for 5 min.**
3. **Transfer the supernatant to a new, clean 1.5 ml microcentrifuge tube. Label the tube containing the pellet as fraction 1. Resuspend the pellet in 100 µl PBS and store on ice.**
4. **Add 100 µl Fractionation Buffer to the supernatant from step 3. Vortex briefly and incubate on ice for 5 min.**
5. **Centrifuge at 14,000 x *g* at 4°C for 5 min.**
6. **Transfer the supernatant to a new, clean 1.5 ml microcentrifuge tube. Label the tube containing the pellet as fraction 2. Resuspend the pellet in 100 µl PBS and store on ice.**
7. **Add 200 µl Fractionation Buffer to the supernatant from step 6. Vortex briefly and incubate on ice for 5 min.**
8. **Centrifuge at 14,000 x *g* at 4°C for 5 min.**

9. **Transfer the supernatant to a new, clean 1.5 ml microcentrifuge tube. Label the tube containing the pellet as fraction 3. Resuspend the pellet in 100 µl PBS and store on ice.**
10. **Add 300 µl Fractionation Buffer to the supernatant from step 9. Vortex briefly and incubate on ice for 5 min.**
11. **Centrifuge at 14,000 x *g* at 4°C for 5 min.**
12. **Transfer the supernatant to a new, clean 2 ml microcentrifuge tube. Label the tube containing the pellet as fraction 4. Resuspend the pellet in 100 µl PBS and store on ice.**
13. **Add 600 µl Fractionation Buffer to the supernatant from step 12. Vortex briefly and incubate on ice for 5 min.**
14. **Centrifuge at 14,000 x *g* at 4°C for 5 min.**
15. **Transfer the supernatant to a new, clean 2 ml microcentrifuge tube. Label the tube containing the pellet as fraction 5. Resuspend the pellet in 100 µl PBS and store on ice.**
16. **Add 20 µl Precipitation Reagent FP1 to the supernatant from step 15. Vortex briefly and incubate on ice for 10 min.**
17. **Add 200 µl Precipitation Reagent FP2 to the supernatant from step 16. Vortex briefly and incubate on ice for 10 min.**
18. **Centrifuge at 14,000 x *g* at 4°C for 15 min.**
19. **Remove and discard the supernatant.**
20. **Resuspend the pellet from step 19 in 1 ml 80% (v/v) acetone which has been stored at –20°C. Vortex and centrifuge at 14,000 x *g* at 4°C for 10 min. Remove and discard the supernatant. Air-dry the pellet.**
21. **Resuspend the pellet in 100 µl 1 x SDS-PAGE sample buffer. Store on ice.**

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications such as 2D-PAGE.

1. **Add four volumes of ice-cold 100% 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

	Comments and Suggestions
Protein assays give inaccurate or inconsistent results	Fractionation Buffer contains components that may interfere with protein quantification assays. A precipitation step (e.g., using acetone, see page 11) to remove interfering substances should be performed before protein assay. Alternatively, the sample can be dialyzed against an appropriate buffer before determination of protein concentration.
A precipitate forms when Fractionation Buffer is stored below room temperature	This precipitate can be dissolved by heating the buffer to 37°C with stirring. Any precipitate should be dissolved before use.
Protease Inhibitor does not thaw at room temperature	Heat the protease inhibitor solution to 37°C with agitation and cool to room temperature.

Notes

Ordering Information

Product	Contents	Cat. no.
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase®	37512
Related products		
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
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: AIL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
Qproteome Cell Compartment Kit	For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase®	37502
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

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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

China
Gene Company Limited
Tel: (852)2896-6283
Fax: (852)2515-9371
E-mail:
Hong Kong:
info@genehk.com
Beijing:
info_bj@genecompany.com
Shanghai:
info_sh@genecompany.com
Chengdu:
gene@public.cd.sc.cn
Guangzhou:
info_gz@genecompany.com

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
PEAI-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
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Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: ventas@valaner.com
Web site: www.valaner.com

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 Saglik 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

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

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