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Qproteome Albumin/IgG Depletion Handbook

For removal of albumin and IgG from human
plasma and serum samples



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Kit Contents

Qproteome Albumin/IgG Depletion Kit (6)	
Catalog no.	37521
Number of preps	6
Albumin/IgG Depletion Spin Columns	6
Luer plugs	10

Storage

Albumin/IgG Depletion Spin Columns should be stored at 2–8°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.

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

Body fluids, such as serum, plasma, and cerebrospinal fluid are widely used in diagnostic procedures. A major problem in analyzing the makeup of these samples is the huge dynamic range of concentrations of their constituent proteins. For example, human serum albumin (HSA) and IgG can amount to 75% of the total protein present. In order to detect and analyze less abundant proteins, removal of these highly abundant proteins is necessary.

Albumin/IgG Depletion Spin Columns are designed for fast and specific removal of albumin and IgG from human serum and plasma samples (Figure 1). The depletion resin in the columns is based on monoclonal antibodies that bind HSA and human IgG with high affinity and specificity. The convenient microspin columns are ready to use. Serum Albumin/IgG Depletion Spin Columns have been optimized for serum or plasma sample sizes up to 25 μ l and allow depletion in just 15 minutes.

Table 1. Typical Albumin/IgG Depletion Spin Column Performance

Recommended sample volume	Up to 25 μ l
Amount of protein applied	1.7–2.0 mg*
Amount of protein recovered	0.5–0.8 mg*

* Results based on 25 μ l sample volume.

Removal of Albumin and IgG from Plasma Samples

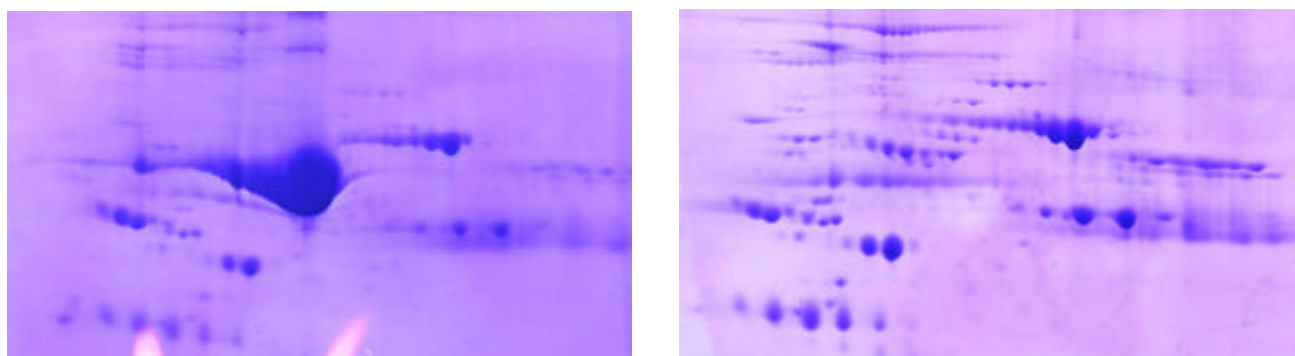
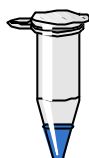


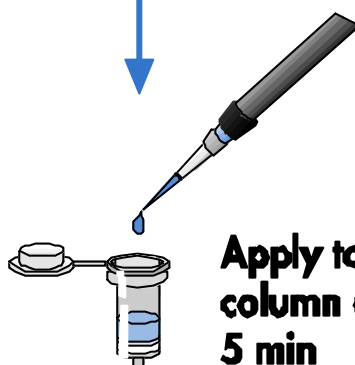
Figure 1 Coomassie[®] stained 2-D PAGE gels showing non-depleted (left) and depleted (right) plasma samples.

Albumin/IgG Depletion Procedure

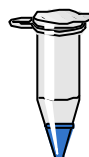
Serum/plasma



Dilute



Apply to depletion column and incubate 5 min



Albumin/IgG depleted serum/plasma proteins

Albumin/IgG Depletion Spin Protocol

To be supplied by user

■ Dilution buffer

PBS (50 mM NaH₂PO₄; 150 mM NaCl, pH 7.2)

Alternative dilution buffer for 2-D PAGE analysis (see step 12):
50 mM Tris·Cl; 4% (w/v) CHAPS; 200 mM urea, pH 7.5

Procedure

- 1. Dilute 25 µl serum or plasma with 75 µl dilution buffer.**
- 2. Centrifuge an Albumin/IgG Depletion Spin Column briefly at 500 x g to remove resin from the screw cap.**
- 3. Remove the screw cap, break off the bottom closure of the spin column, and drain the storage buffer by gravity flow.**
- 4. Equilibrate the spin column by pipetting 2 x 0.5 ml aliquots of dilution buffer onto the spin column and letting each run out by gravity flow.**
- 5. Close the spin column with a luer plug.**
- 6. Apply the sample prepared in step 1 onto the column.**
- 7. Close the lid of the spin column and shake vigorously to obtain a homogenous suspension. Incubate for 5 min on an end-over-end shaker at room temperature (15–25°C).**
- 8. Remove the luer plug and transfer the spin column to a clean centrifuge tube.**
- 9. Loosen the cap of the column a quarter turn.**
This is necessary to avoid a vacuum inside the spin column.
- 10. Collect the flow-through by centrifugation at 500 x g for 10 s.**
The flow-through contains the depleted protein sample.
- 11. Wash the column with 2 x 100 µl aliquots of dilution buffer, collecting each wash fraction by centrifugation at 500 x g for 10 s.**
- 12. Combine the flow-through fraction from step 10 and the two wash fractions from step 11.**
Before analysis using 2-D PAGE it is necessary to concentrate and desalt the depleted samples by acetone precipitation (see page 9).
If using the alternative dilution buffer samples can be used directly for 2-D PAGE without a desalting step. It should however be noted that plasma contains significant amounts of salt, which increases the current during IEF.
- 13. Optional: To recover the maximum amount of unbound protein additional wash steps of 2 x 500 µl can be performed.**

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 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

Insufficient depletion of albumin and IgG

- | | |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| a) Sample and resin were not mixed before incubation | Make sure that the resin and the sample form a homogenous suspension before incubation on the end-over-end shaker. |
| b) Non-human samples were used | The Albumin/IgG Depletion Spin Columns are specific for human samples. |
| c) Recommended buffer not used | The Albumin/IgG Depletion Spin Columns are optimized for samples diluted in PBS or the indicated alternative buffer. |

High conductivity in isoelectric focusing (IEF)

- | | |
|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Buffer or sample contained high concentration of salt | <p>PBS contains 150 mM NaCl which gives rise to high conductivity in IEF. Desalting by acetone precipitation (see above) or dialysis must be performed before IEF analysis.</p> <p>Although the alternative dilution buffer does not contain any NaCl, the serum and plasma samples themselves contain significant amounts of salt which may have to be removed prior to IEF.</p> |
|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Ordering Information

Product	Contents	Cat. no.
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
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: ALL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
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 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

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