February 2009

Albumin and IgG Depletion Handbook

Qproteome® Murine Albumin Depletion Kit

Qproteome Albumin/IgG Depletion Kit

Qproteome Albumin/IgG Depletion Plate

Albumin/IgG Depletion Cartridge

Albumin Affinity Cartridges

For separation of albumin and IgG from plasma and serum samples

QIAGEN®

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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.

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- microRNA research and RNAi
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Qproteome Murine Albumin Depletion Kit	**(6)**
Catalog no.	**37591**
Number of preps	**6**
Murine Albumin Depletion Spin Columns	6
Luer plugs	10
Handbook	1

Qproteome Albumin/IgG Depletion Kit	**(6)**
Catalog no.	**37521**
Number of preps	**6**
Albumin/IgG Depletion Spin Columns	6
Luer plugs	10
Handbook	1

Qproteome Albumin/IgG Depletion Plate	
Catalog no.	**37009**
Number of preps	**96**
Albumin/IgG Depletion Plate	1
Handbook	1

Albumin/IgG Depletion Cartridge	**(3 x 1 ml)**
Catalog no.	**37003**
Albumin/IgG Depletion Cartridge	3
Handbook	1

Albumin Affinity Cartridge	**(3 x 1 ml)**
Catalog no.	**37013**
Albumin Affinity Cartridge	3
Handbook	1

Storage

Qproteome Depletion products should be stored at 2–8°C. For longer storage, or after use, cartridges should be stored in PBS supplemented with 0.05% (w/v) sodium azide.

Product Use Limitations

Qproteome Depletion products are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor has they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of these products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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. 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 Depletion products 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/Support 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/support/MSDS.aspx 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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Qproteome Depletion products is tested against predetermined specifications to ensure consistent product quality.

Introduction

Qproteome Depletion Spin Columns and Plates and Albumin Affinity Cartridges are designed for fast and specific removal of albumin and IgG from serum and plasma samples (Table 1). The removal of these highly abundant proteins greatly facilitates analysis of less abundant proteins and biomarkers (Figure 1). The depletion resin used in these products is based on monoclonal antibodies that bind albumins and immunoglobulins with high affinity and specificity.

Body fluids, such as serum, plasma, and cerebrospinal fluid are widely used in life science applications. A major problem in analyzing the makeup of these samples is the huge, dynamic range of concentrations of their constituent proteins. For example, serum albumin 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.

Table 1. QIAGEN albumin depletion products

	Species	Sample size	Processing
Qproteome Albumin/IgG Spin Columns	Human	Up to 25 µl plasma or serum	Centrifuge or QIAcube®
Qproteome Murine Albumin Spin Columns	Mouse or rat	Up to 25 µl plasma or serum	Centrifuge or QIAcube
Qproteome Albumin/IgG Depletion Plate	Human	96 x 5 µl plasma or serum	Centrifuge with swing-out rotor and 96-well plate adapter
Albumin Affinity Cartridges	Human, mouse, or rat	Up to 150 µl plasma or serum	FPLC™ system (e.g., ÅKTA™, BioLogic™) or syringe
Albumin/IgG Depletion Cartridges	Human, mouse, or rat	Up to 150 µl plasma or serum	FPLC system (e.g., ÅKTA, BioLogic) or syringe

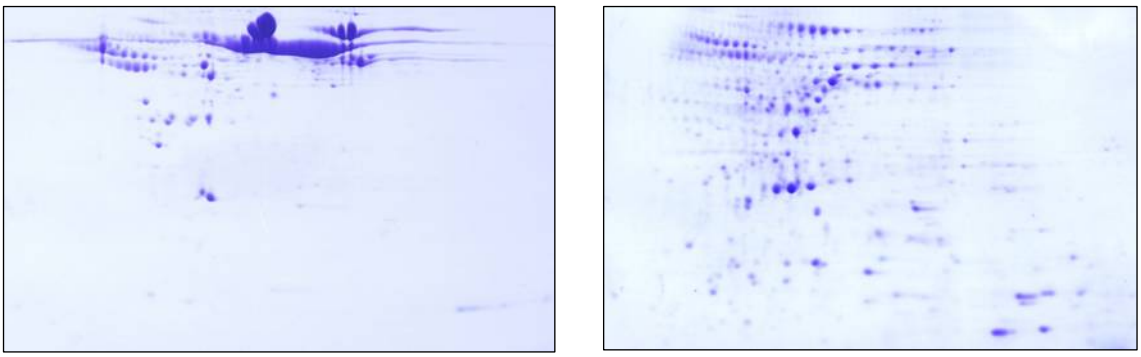


Figure 1 Removal of albumin from rat plasma samples. Coomassie® stained 2D PAGE gels showing nondepleted (left) and depleted (right) rat plasma samples.

Principle and Procedures

Diluted serum or plasma is mixed with or passed through an affinity resin comprising antibodies against IgG and/or albumin covalently attached to an inert sepharose matrix. Immunoglobulins and/or albumin bind to the resin with high affinity and the flow-through fraction contains the depleted sample. The resin is then washed to ensure that all proteins not associated with albumin are recovered. The resin can also be washed using buffers of varying stringency to investigate proteins that associate with albumin (the so-called "albuminome").

Protocols in this handbook

This handbook contains protocols for:

- Manual depletion of serum or plasma using Qproteome Albumin/IgG or Murine Albumin Spin Columns — see page 11
- Manual depletion of serum or plasma using Qproteome Albumin/IgG Depletion Plates — see page 13
- Depletion of serum or plasma using Albumin Affinity Columns or Albumin/IgG Depletion Columns and a liquid chromatography system — see page 15

Automated depletion of serum and plasma

The QIAcube can be used together with 2 Qproteome Depletion Spin Column Kits for fully automated depletion of serum or plasma. Twelve samples are processed simultaneously, and protocols can be easily downloaded from the QIAcube Web portal at www.qiagen.com/myQIAcube.

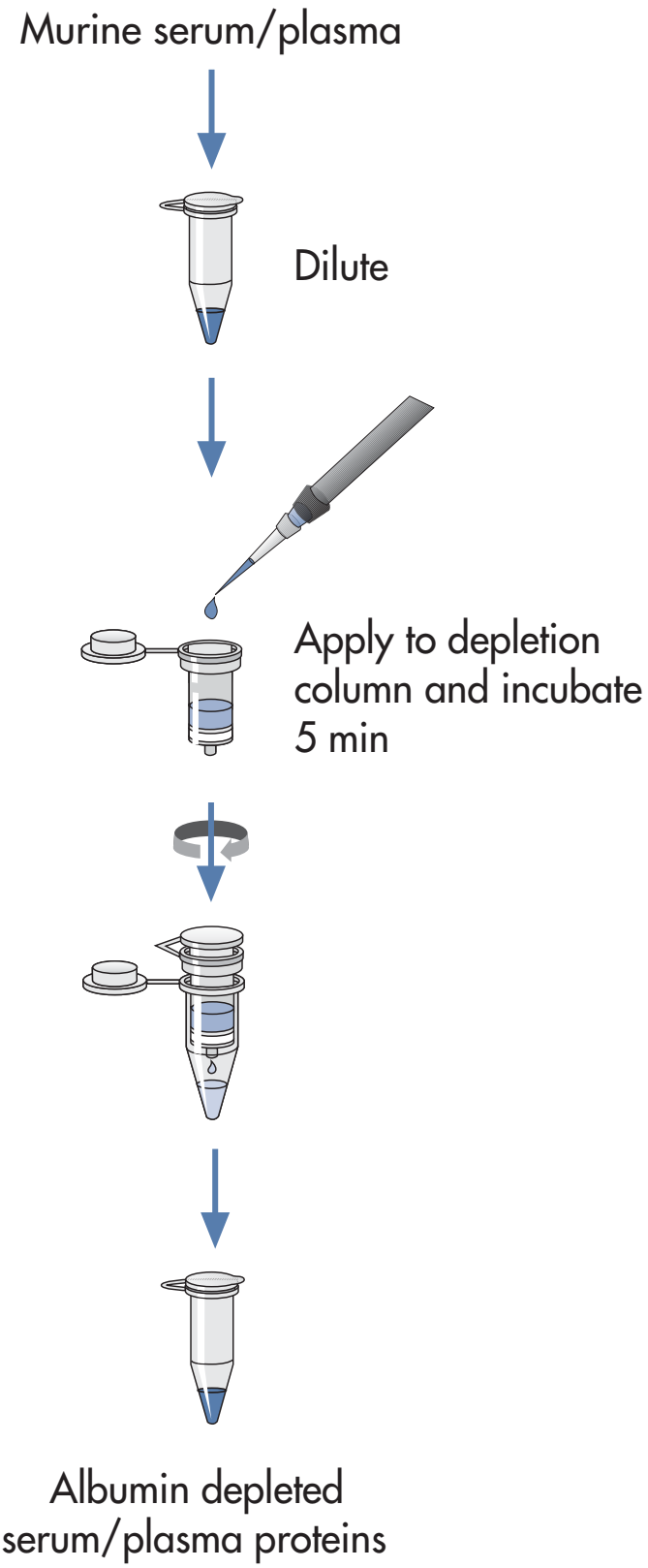
Albumin Affinity and Albumin/IgG Depletion Cartridge connections

Albumin Affinity and Albumin/IgG Depletion Cartridges can be used for depletion of serum or plasma in an automated procedure using a chromatography system (such as the ÄKTAdesign or FPLC System). The cartridge inlet and outlet dimensions and required connectors and adapters for manual and automated procedures are detailed in the table below.

Table 2. Connectors required for Albumin Affinity Cartridges

	Inlet	Outlet
Albumin Affinity and Albumin/IgG Depletion Cartridge	1/16″ female (ÄKTAdesign)	M6 male (FPLC)
Adapters for manual procedure using a syringe	1/16″ male/Luer female (e.g., G.E Healthcare Product Code 18-1112-51)	
Connector for automated procedure (ÄKTAdesign 1/16″ connectors)	No adapter required	Union M6 female / 1/16″ female (e.g., G.E Healthcare Product Code 18-1123-94)
Connector for automated procedure (M6 fittings, [FPLC])	Union M6 female / 1/16″ male (e.g., G.E Healthcare Product Code 18-3858-01)	SRTC-2, M6 female (0.5 mm i.d.) (e.g., G.E Healthcare Product Code 18-3856-01)

Albumin Depletion Procedure



Protocol: Albumin/IgG Depletion Spin Column Protocol

This protocol is suitable when performing manual depletion of serum or plasma using Qproteome Albumin/IgG or Murine Albumin Spin Columns.

Equipment and reagents to be supplied by user

- PBS (50 mM NaH_2PO_4; 150 mM NaCl, pH 7.2)*
- Alternative dilution buffer for 2D PAGE analysis (see step 12): 50 mM Tris·Cl; 4% (w/v) CHAPS; 200 mM urea, pH 7.5*
- End-over-end shaker

Procedure

1. **Dilute 25 µl serum or plasma with 75 µl PBS buffer.**
2. **Centrifuge a Qproteome 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 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 spin column with 2 x 100 µl aliquots of PBS buffer, collecting each wash fraction by centrifugation at 500 x *g* for 10 s.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

12. **Combine the flow-through fraction from step 10 and the two wash fractions from step 11.**

    Before analysis using 2D PAGE, it is necessary to concentrate and desalt the depleted samples by acetone precipitation (see page 17).

    If using the alternative dilution buffer, samples can be used directly for 2D PAGE without a desalting step. It should however be noted that plasma contains significant amounts of salt, which increases the current during IEF and may need to be removed.

13. **Optional: To recover the maximum amount of unbound protein additional wash steps using 2 x 500 µl PBS buffer can be performed.**

Protocol: Albumin/IgG 96-Well Depletion Protocol

This protocol is suitable when performing manual depletion of serum or plasma using Qproteome Albumin/IgG Depletion Plates.

Equipment and reagents to be supplied by user

- PBS (50 mM NaH_2PO_4; 150 mM NaCl, pH 7.2)*
- Alternative dilution buffer for 2D PAGE analysis (see step 5): 50 mM Tris·Cl; 4% (w/v) CHAPS; 200 mM urea, pH 7.5*
- 96-Well Microplates RB (QIAGEN cat. no. 19581)
- Microplate shaker
- Centrifuge for 96-well plates (e.g., Sigma 4K-15)

Things to do before starting

- Dilute plasma or serum samples by adding 3 parts PBS buffer to 1 part serum or plasma (e.g., add 15 µl PBS to 5 µl serum or plasma to give a final sample volume of 20 µl).

Procedure

1. **Take the depletion plate out of the bag, and remove the tape sheets from the top and bottom of the plate.**

   When handling the plate ensure that it remains horizontal. It is easier to remove the tape from the bottom first.

2. **To remove the storage buffer, place the depletion plate on the top of a 96-well microplate and centrifuge at 100 x *g* for 2 min and discard the flow-through.**
3. **Equilibrate the resin by placing the depletion plate on top of the 96-well microplate, adding 150 µl PBS buffer to each well, and centrifuging at 100 x *g* for 2 min. Discard the flow-through and repeat.**
4. **Place the depletion plate on top of a clean 96-Well Microplate RB and add 20 µl diluted plasma (1 part plasma or serum + 3 parts PBS buffer) to each well and incubate the plate by shaking on a microplate shaker for 5 min.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

5. **Place the depletion plate (together with the 96-well microplate) into the centrifuge and centrifuge at 2000 x *g* for 30 s.**

   The flow-through contains the depleted sample.

   Before analysis using 2D PAGE, it is necessary to concentrate and desalt the depleted samples by acetone precipitation (see page 17).

   If using the alternative dilution buffer, samples can be used directly for 2D PAGE without a desalting step. It should however be noted that plasma contains significant amounts of salt, which increases the current during IEF and may need to be removed.

6. **Optional: Increase recovery of unbound proteins by performing up to three sequential washes using 20 µl PBS buffer per well and centrifuging at 2000 x *g* for 30 s.**

Protocol: Depletion Using a Depletion Cartridge and an Automated Chromatography System

This protocol is suitable for depleting up to 150 µl serum or plasma using a liquid chromatography system (such as the ÄKTAdesign or FPLC System). During equilibration, loading, washing, and elution monitor the back pressure generated by the chromatography system. Do not allow back pressure to exceed 5 bar (0.5 MPa).

Equipment and reagents to be supplied by user

- PBS buffer (50 mM NaH_2PO_4; 150 mM NaCl, pH 7.2)*
- Alternative dilution buffer for 2-D PAGE analysis (see step 5): 50 mM Tris·Cl; 4% (w/v) CHAPS; 200 mM urea, pH 7.5*
- **Optional**: Elution Buffer (20 mM glycine, pH 2.0)
- Connector adapter(s) (see Table 2, page 9)

Things to do before starting

- Dilute plasma or serum samples by adding 3 parts PBS buffer to 1 part serum or plasma (e.g., add 300 µl PBS to 100 µl serum or plasma to give a final sample volume of 400 µl).
- Immediately before applying to column, filter-sterilize (0.2 or 0.45 µm) or centrifuge the sample (≥ 10,000 x *g*) to ensure that it is particle-free.

Procedure

1. **Fill system pumps with PBS buffer and attach pump to the cartridge inlet taking care not to introduce air into the system.**
2. **Remove cartridge outlet stopper and attach to the system tubing.**
3. **Equilibrate the cartridge with 10 column volumes (10 ml) of buffer.** Use a flow rate of 1ml/min.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

4. **Using a syringe or a superloop, load the diluted sample onto the cartridge and wash until the A_{280} returns to the baseline value. Collect the flow-through fraction. Use a reduced flow rate of 0.1 ml/min to ensure efficient binding.**

   The flow-through fraction is the albumin/IgG-depleted sample.

   Before analysis using 2D PAGE, it is necessary to concentrate and desalt the depleted samples by acetone precipitation (see page 17).

   If using the alternative dilution buffer, samples can be used directly for 2D PAGE without a desalting step. It should however be noted that plasma contains significant amounts of salt, which increases the current during IEF.

5. **Optional: Fill system pumps with Elution Buffer and wash the cartridge until the A_{280} returns to the baseline value with a flow rate of up to 1 ml/min.**

   The eluate fraction contains IgG and/or albumin and associated proteins.

6. **Fill system pumps with PBS and equilibrate the cartridge until pH is neutral.**

   Use at least 10 column volumes (10 ml). For longer term storage, supplement the equilibration buffer with 0.1% sodium azide.

Protocol: Acetone Precipitation of Protein Fractions

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

Procedure

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 precooled 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

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. 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
Spin columns — 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) Sample originated from wrong species	The Murine Albumin Depletion Spin Columns are specific for murine samples, and Albumin/IgG Depletion Spin Columns are specific for human samples.
c) Recommended buffer not used	The Murine Albumin Depletion Spin Columns are optimized for samples diluted in PBS or the indicated alternative buffer.
Plate — Insufficient depletion of albumin and IgG	
a) Sample and resin were not mixed during incubation	Make sure that the resin and the sample form a homogenous suspension during incubation on the shaker.
b) Sample originated from wrong species	The Albumin/IgG 96 Depletion Plate is specific for human samples.
c) Recommended buffer not used	The Albumin/IgG 96 Depletion Plate is optimized for samples diluted in PBS or the indicated alternative buffer.
FPLC cartridges — Insufficient depletion of albumin and IgG	
a) Sample was applied at a high flow rate	Use a flow rate of 0.1 ml/min to load the sample.

	Comments and suggestions
b) Sample originated from wrong species	Albumin Affinity and Albumin/IgG Depletion Cartridges are specific for human, rat, and murine samples.
c) Recommended buffer not used	The Albumin/IgG Depletion Cartridges 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	PBS contains 150 mM NaCl which gives high conductivity in IEF. Desalting by acetone precipitation (see above) or dialysis must be performed before IEF analysis. Although the alternative dilution buffer does not contain NaCl, the serum and plasma samples themselves contain significant amounts of salt which may have to be removed prior to IEF.

Ordering Information

Product	Contents	Cat. no.
Qproteome Murine Albumin Depletion Kit	For albumin depletion of 6 murine serum or plasma samples: Murine Albumin Depletion Spin Columns (6)	37591
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
Qproteome Albumin/IgG Depletion Plate	For albumin depletion of 96 murine serum or plasma samples: Murine Albumin Depletion Plate (1)	37009
Albumin Affinity Cartridge (3 x 1 ml)	For albumin depletion of human or murine serum or plasma samples: 3 cartridges prefilled with 1 ml Albumin Affinity resin	37013
Albumin/IgG Depletion Cartridge (3 x 1 ml)	For albumin/IgG depletion of human or murine serum or plasma samples: 3 cartridges prefilled with 1 ml Albumin/IgG Depletion resin	37003
Centrifuge 4K15C (220 V, 50 Hz)	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81220
Centrifuge 4-15C (220 V, 50 Hz)	Universal laboratory centrifuge with brushless motor (220 V, 50 Hz)	81020

Qproteome depletion products are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products.

Notes

Notes

Limited License Agreement

Use of these products signifies the agreement of any purchaser or user of the Qproteome Albumin/IgG Depletion Kit or Qproteome Murine Albumin Depletion Kit or Albumin Affinity Cartridges, or Qproteome Albumin/IgG Depletion Plate to the following terms:

1. The Qproteome Albumin/IgG Depletion Kit or Qproteome Murine Albumin Depletion Kit or Albumin Affinity Cartridges, or Qproteome Albumin/IgG Depletion Plate may be used solely in accordance with the *Albumin and IgG Depletion Handbook* and for use with components contained in the kits only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of these products with any components not included within these products except as described in the *Albumin and IgG Depletion Handbook* and additional protocols available at www.qiagen.com.
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Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

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