Proteome Purify™ 12

Human Serum Protein Immunodepletion Resin

Catalog Number IDR012-020
IDR012-040

For the removal of twelve high-abundance proteins from human serum or plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Progress in the field of proteomics has enabled the identification of more than 1000 discrete proteins in plasma (1, 2). It is likely that all proteins, whether they are primarily secreted or present in cytoplasm, organelles, the cytoskeleton, or even the membrane, are represented in the plasma in some amount (1). The promise of proteomics lies in detecting proteins that show over- or under-representation in disease states and can then be used as markers of various conditions (1). Plasma is easy to obtain and is a likely source of such markers. The large dynamic range of protein concentrations present in serum, plasma, or other potential samples for proteomics represents a major challenge for analysis of the proteome (1-5). The range of serum or plasma protein concentrations from low-abundance proteins, such as cytokines (pg/mL), to highly abundant proteins such as albumin (30-50 mg/mL), represents at least ten orders of magnitude. Analysis by even the most sensitive of methods for proteomics greatly benefits from removal of the proteins of highest abundance which interfere with detection of proteins present in low abundance (1-5).

The Human Proteome Organization (HUPO) and others have identified depletion of high-abundance proteins as an essential first step in effective proteome analysis and the use of antibodies as the most effective means to achieve this depletion (2-4). Collectively, the proteins listed in the table below constitute approximately 95% of all plasma proteins. The removal of these twelve high-abundance proteins by a high-capacity method is likely to reduce the dynamic range of plasma protein concentrations by nearly three orders of magnitude. Antibody-mediated depletion is superior to other methods when compared for reproducibility and ease of preparation (3, 5). These factors are critical when analyzing low-abundance proteins since an easier preparation protocol allows higher throughput, while good reproducibility is essential for determining disease marker status.

R&D Systems Proteome Purify 12 Human Serum Protein Immunodepletion Resin provides an easy-to-use, high capacity system to reproducibly remove these twelve high-abundance proteins from human serum or plasma.

Protein
α_1-Acid Glycoprotein
α_1-Antitrypsin
α_2-Macroglobulin
Albumin
Apolipoprotein A-I
Apolipoprotein A-II
Fibrinogen
Haptoglobin
IgA
IgG
IgM
Transferrin

PRINCIPLE OF THE ASSAY

The Proteome Purify 12 Human Serum Protein Immunodepletion Resin is an innovative method for preparing serum or plasma for two-dimensional electrophoresis (2DE) analysis. The twelve proteins removed by this kit represent approximately 95% of the protein contained in serum and plasma. Albumin and IgG alone account for more than 60% of the total protein. The relative concentration of the high-abundance proteins masks the presence of the other proteins with similar isoelectric points and/or molecular weight.

There are two major advantages to removing high-abundance proteins prior to 2DE or other types of proteomic analysis.

- Removal allows visualization of proteins that co-migrate or co-purify with the high-abundance proteins.
- Removal of high-abundance proteins in the sample allows one to increase the protein load or concentration, improving visualization or analysis.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.

PRECAUTIONS

The Immunodepletion Resin contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # IDR012-020	CATALOG # IDR012-040	DESCRIPTION	STORAGE CONDITIONS
Immunodepletion Resin	893677	1 bottle	2 bottles	20 mL/bottle of 50/50 (v/v) slurry of a proprietary absorption gel in PBS with 0.02% sodium azide (pH 7.4).	Store at 2-8 °C.*
Spin-X® Filter Units	895933	4 filters	4 filters	Centrifuge tube filters with a 0.22 µm cellulose acetate membrane.	Store at room temperature.

IDR012-020 contains sufficient resin to run 20 tests.
IDR012-040 contains sufficient resin to run 40 tests.

This product is available in bulk. Please contact your sales representative or distributor.

OTHER SUPPLIES REQUIRED

- Microcentrifuge capable of operating at 1000-2000 x g
- Microcentrifuge tubes
- Adjustable pipette and pipette tips
- Test tubes (16 x 100 mm)
- Rotary shaker or mixer
- 5000 Da MWCO spin concentrator (optional; Sigma, Catalog # Z614009)
- Spin-X Centrifuge Tube Units (R&D Systems, Catalog # SPINX8160), or equivalent

PROCEDURE

Bring Immunodepletion Resin to room temperature before use.
Perform procedure at room temperature.

Note: *The concentration of serum/plasma proteins can vary widely depending on the origin of the sample. The concentration of certain proteins are known to rise due to stress, infection, inflammation, and other factors (6). Due to these factors users may need to adjust loading volumes.*

1. Add 10 µL of serum or plasma to a test tube.

   Note: *It is essential that the Immunodepletion Resin be homogeneous prior to pipetting.*

2. Add 1.0 mL of the suspended Immunodepletion Resin to the test tube containing the sample.
3. Mix the Immunodepletion Resin on a rotary shaker for 30-60 minutes. The mixing speed should be sufficient to keep the Immunodepletion Resin in suspension.
4. After the incubation period, pipette equal amounts of the Immunodepletion Resin into the upper chamber of two Spin-X Filter Units.
5. Centrifuge for 2 minutes at 1000-2000 x g in a microcentrifuge tube. The volume of the combined filtrates will be approximately 400-500 µL.
6. The sample is now ready for further processing or may be stored for later use.
7. Discard the used Immunodepletion Resin.

SAMPLE ANAYLSIS

Sample processing will depend on the type of analysis that will be done.

Initial depletion of the serum or plasma will result in the removal of greater than 90% of the two high abundance proteins. If higher levels of depletion are required, the sample should be concentrated (5000 Da MWCO spin concentrator) and depleted a second time.

The depleted sample will be in a buffer of PBS with 0.02% sodium azide. Spin concentrators are recommended if desalting is required.

A number of protocols are available for acetone precipitation or acetone/TCA precipitation. A protocol for acetone precipitation is described on the next page.

ACETONE PRECIPITATION

1. Add one volume of depleted sample to an acetone safe test tube.
2. Add 5 volumes of cold (-20 °C) 100% acetone to the sample and store at ≤ -20 °C overnight.
3. Centrifuge at 15,000 x g for 30 minutes at 2-8 °C.
4. Decant the supernate and replace with an equal volume of cold 50% acetone. Vortex to resuspend the pellet.
5. Centrifuge at 15,000 x g for 30 minutes at 2-8 °C.
6. Decant the supernate and replace with an equal volume of cold 50% acetone. Vortex to resuspend the pellet.
7. Centrifuge at 15,000 x g for 30 minutes at 2-8 °C.
8. Allow the pellet to air dry for 24 hours at room temperature.
9. Dissolve the pellet in an appropriate buffer.

REFERENCES

1. Anderson, N.L. *et al.* (2004) Mol. Cell. Proteomics **3**:311.
2. Omenn, G.S. *et al.* (2005) Proteomics **5**:3226.
3. Whiteaker, J.R. *et al.* (2007) J. Proteome Res. **6**:828.
4. Zolotarjova, N. *et al.* (2005) Proteomics **5**:3304.
5. Liu, T. *et al.* (2006) Mol. Cell. Proteomics **5**:2167.
6. Henry, J.B. (1996) in *Clinical Diagnosis and Management by Laboratory Methods*, McPherson, R. and M. Pincus eds., Sanders, ISBN-13978-0721688640.

NOTES