MULTIPLE AFFINITY REMOVAL SYSTEM (MARS)

Any molecule that can be bound effectively by an antibody can be purified. Purified antibodies are coupled to the inert solid phase and mixed with the antigen solution under conditions that favor adsorption. Following antigen capture, unwanted antigens are removed by washing, and the purified antigen is released by switching to conditions that favor desorption.

The ideal antibody should possess two properties:

(a) High intrinsic affinity, since an antibody attached to a solid phase has no room for cooperative binding.

(b) Ease of elution: This depends on the type and number of antigen-antibody bonds: the fewer types of interactions involved, the easier all of them can be destabilized.

Supports and Support Activation

Rigid

Uniform size- Narrow size distribution

Hydrophilic

Nontoxic

Stable

Matrix should withstand these extremes of pH

Readily derivatized

Solid matrix

The matrix should be easily modified for antibody attachment, and should be macroporous with uniform particle and pore size and good flow properties

A compromise should be achieved between pore size and surface area, as supports with small pore size have a large surface area, much of which may not be available for immobilization of antibody

In contrast, large pore support systems do not have accessibility problems, but may result in a low level of antibody attachment due to the small surface area.

Supports with pore sizes of 300-500A° which is approximately three to five times the diameter of an antibody, allow for maximum antibody coverage, as well as for suitable binding of immobilized antibodies to many small or medium sized targets

Low performance supports

beads of carbohydrate-related materials or synthetic organic supports

The main disadvantage of these materials is their slow mass transfer properties and their limited stability at high flow rates and pressures

Carbohydrate related materials

Fibrous cellulose — poor flow characteristics of the medium.

Beaded agarose — it does not have the required mechanical and chemical stability

Synthetic organic supports

acrylamide polymers, copolymers or derivatives

polymethacrylate derivatives

polyethersulfone matrices

High efficiency supports

Derivatized silica, glass

polystyrene based perfusion media

POROS is a polystyrene/ divinylbenzene polymer with both large (permit through) and small (permit diffusion) pores

azalactone beads

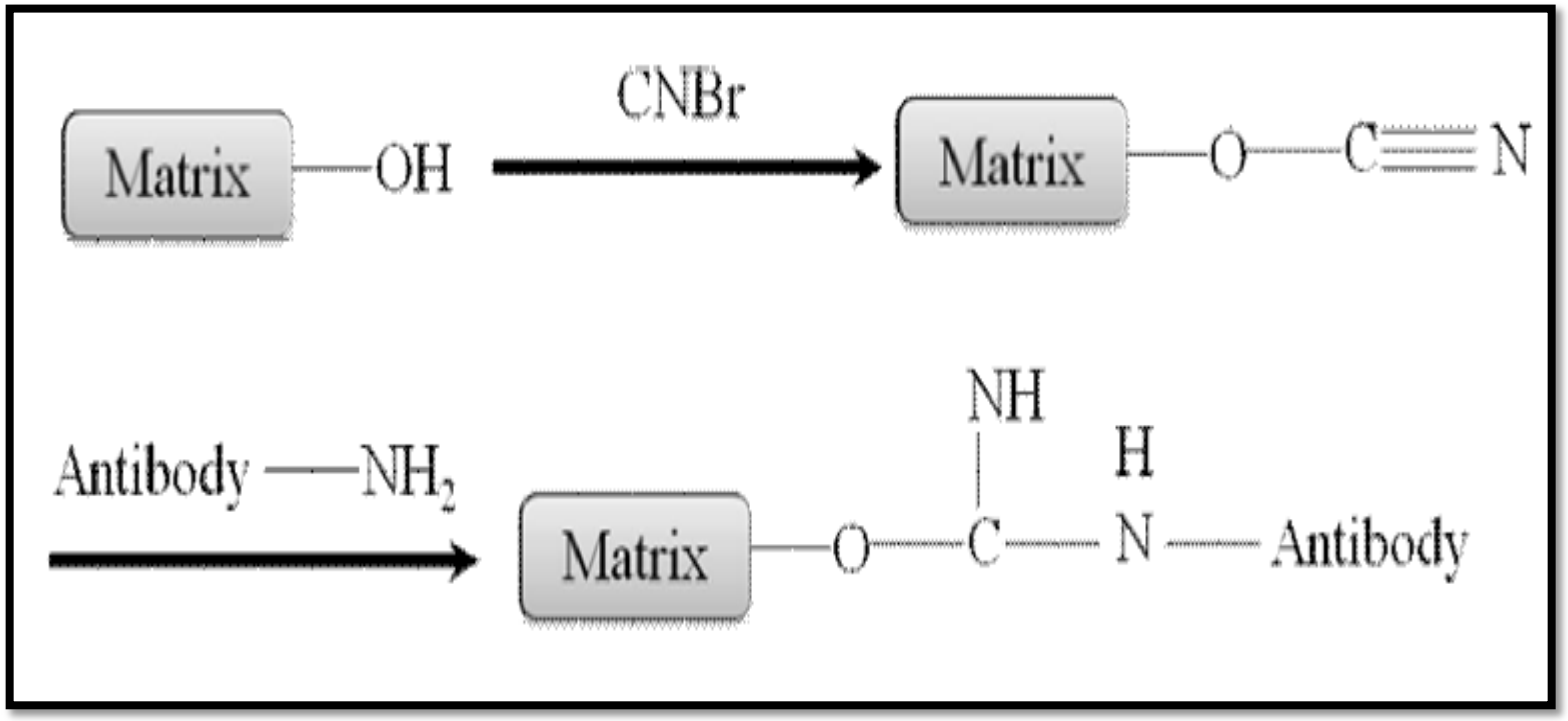
These matrices include:

1. carbohydrate-based media (agarose, dextrose, or cellulose),
2. synthetic organic supports such as acrylamide polymers, polymethacrylate derivatives, polyethersulfone matrices, or
3. inorganic materials such as silica and zirconia

Cross-linked agarose is an extremely popular matrix because it can usually withstand a wide pH range (e.g. pH 3–12), most aqueous solvents (including denaturants), many organic solvents or modifiers, and enzymatic treatments.
However, agarose beads and other soft gel matrices are more susceptible to pressure, relative to stronger supports, such as silica, polystyrene and other highly cross-linked materials

Antibodies are immobilized on the support.

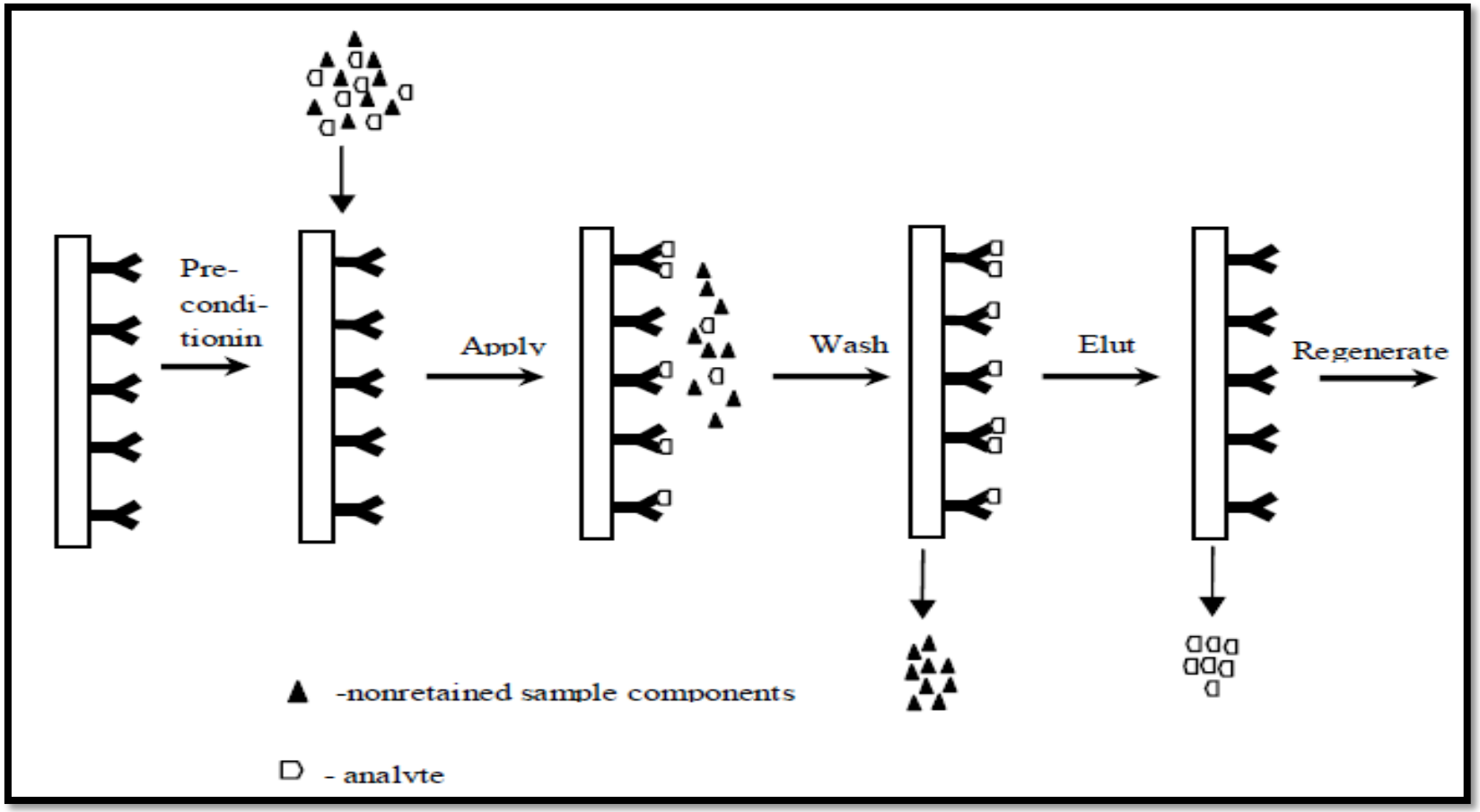
The most commonly used activation method for polysaccharide matrices employs cyanogen bromide.



For instance, if 1 ml of CNBr-activated Sepharose slurry is derivatized with 5 mg of antibody (a typical ratio) and if one in three antibodies remains fully active (and can thus bind two antigen molecules), its binding capacity for a 25 kDa molecule is still approximately 500 µg per run.

Antibodies can also be immobilized onto a matrix by using a secondary ligand. In this case, biotinylated antibodies are adsorbed to a support containing immobilized avidin, streptavidin, or neutravidin.

System consists of a Multiple Affinity Removal System Column based on affinity interactions and optimized buffers for sample loading, washing, eluting, and regenerating.



Affinity Removal System helps to unlock the potential of the human and mouse serum proteome for biomarker or drug target discovery applications in less than 30 minutes.

Identifying proteins or peptides from human/mouse serum and plasma samples is challenging because targets of most interest are often masked by high-abundance proteins of limited interest

Affinity Removal System eliminates the masking effect of highly abundant proteins in serum, plasma, or CSF samples so that you can easily detect lower-abundant proteins and peptides

based on affinity interactions and optimized buffers for sample loading, washing, eluting, and regenerating

Features

the loading capacity

Process large amounts of plasma

Minimize sample loss

Increases detection limits of protein separation methods such as 1DGE, 2DGE and LC/MS

The Agilent Human 14 Multiple Affinity Removal System

albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, and transthyretin

Six highly abundant proteins removed from human serum

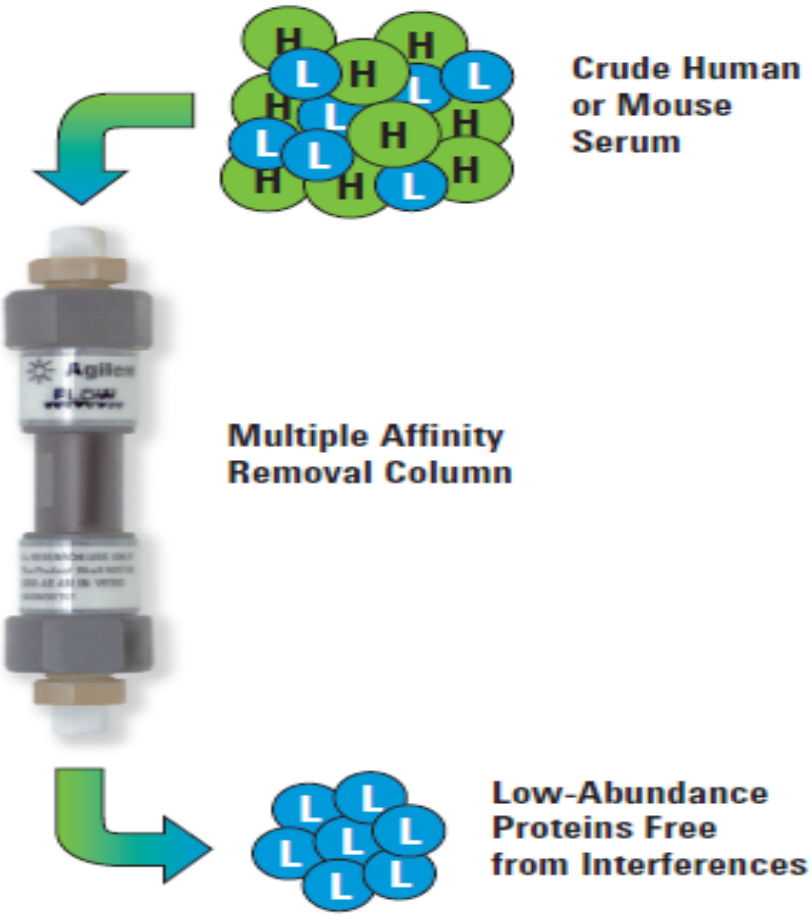
- Albumin
- IgG
- Antitrypsin
- IgA
- Transferrin
- Haptoglobin

the Ms-3 column

- Albumin
- IgG
- Transferrin appropriate for rat serum

These proteins cover 80 to 90 percent of total serum protein mass. Removing them **allows you to load up to 10 times more low-abundance protein mass** onto gels or LC/MS for analysis.

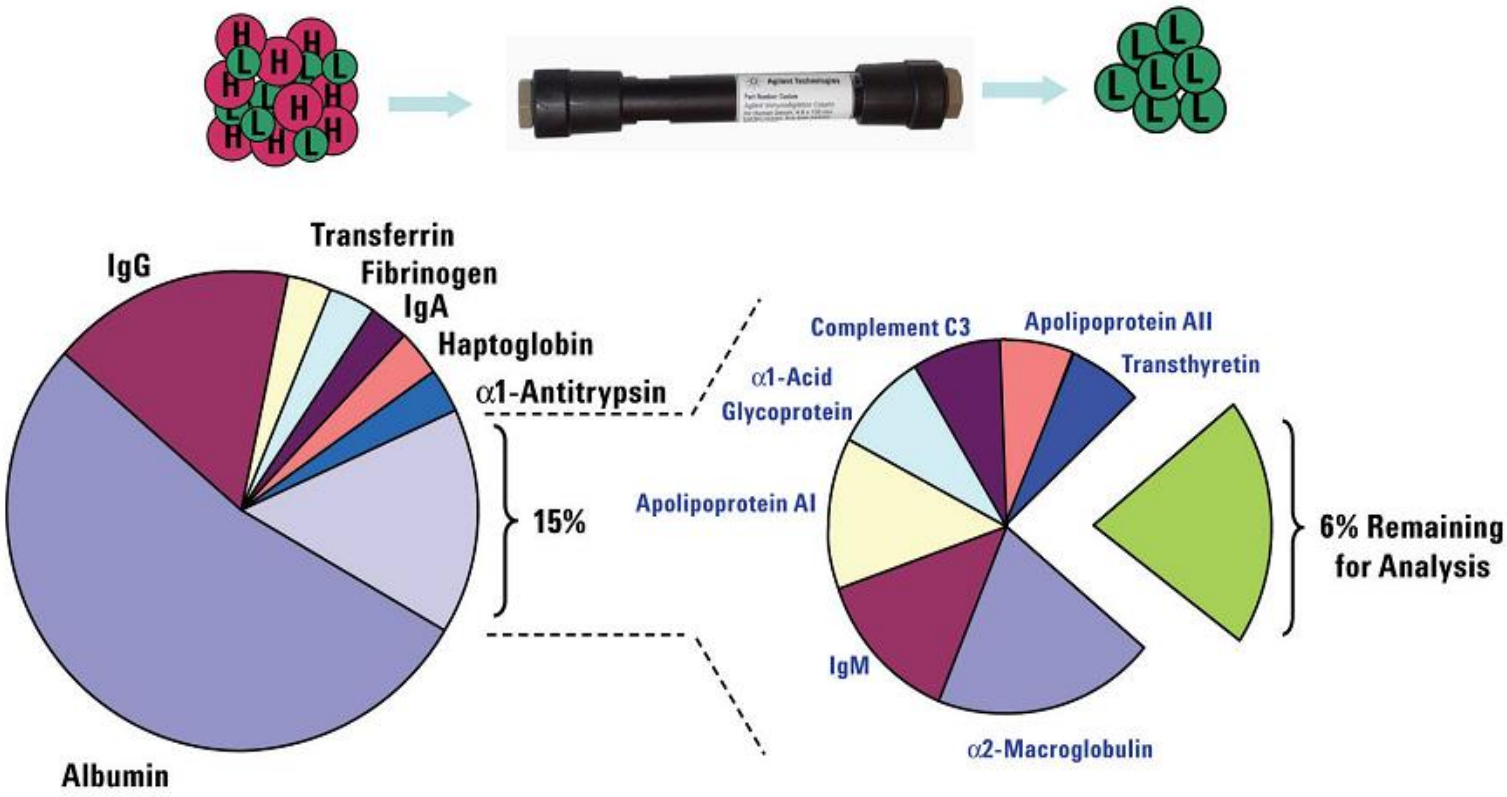
depletes approximately 94% of total protein mass from human serum



H **High Abundance Proteins** (Human: Albumin, IgG, IgA, Transferrin, Haptoglobin, Antitrypsin; Mouse: Albumin, IgG, Transferrin)

L **Low-Abundance Proteins** (Biomarkers for disease and drug targets)

- The Multiple Affinity Removal System consists of a Multiple Affinity Removal Column based on antibody-antigen interactions and optimized buffers for sample loading, washing, eluting and regenerating



Multiple Affinity Removal System Human 14.

Multiple Affinity Removal System Selection Guide

Product	Proteins Removed	Total Protein Removed	Dimension	Load Capacity	Part No.
MARS Human-14	Albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin	94%	Spin Cartridge	8-10 µL	5188-6560
			4.6 x 50 mm	20 µL	5188-6557
			4.6 x 100 mm	40 µL	5188-6558
			10.0 x 100 mm	250 µL	5188-6559
MARS Human-7	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen	88-92%	Spin Cartridge	12-14 µL	5188-6408
			4.6 x 50 mm	30-35 µL	5188-6409
			4.6 x 100 mm	60-70 µL	5188-6410
			10.0 x 100 mm	250-300 µL	5188-6411
MARS Human-6	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	7-10 µL	5188-5230
			4.6 x 50 mm	15-20 µL	5185-5984
			4.6 x 100 mm	30-40 µL	5185-5985
MARS Human-6 High Capacity	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	14-16 µL	5188-5341
			4.6 x 50 mm	30-40 µL	5188-5332
			4.6 x 100 mm	60-80 µL	5188-5333
			10.0 x 100 mm	up to 340 µL	5188-5336
MARS Human-2	Albumin, IgG	69%	Spin Cartridge	50 µL	5188-8825
			4.6 x 50 mm	100 µL	5188-8826
MARS Human-1	Albumin	50-55%	Spin Cartridge	65 µL	5188-5334
			4.6 x 50 mm	130 µL	5188-6562
MARS Mouse-3	Albumin, IgG, transferrin	80%	Spin Cartridge	25-30 µL	5188-5289
			4.6 x 50 mm	37-50 µL	5188-5217
			4.6 x 100 mm	75-100 µL	5188-5218

QTY	Part Number	Description	Unit	Price
	5188-6559	Multiple Affinity Removal Column, 10 x 100 mm, Hu-14		28,577.00 USD
	5188-6558	Multiple Affinity Removal Column, 4.6 x 100 mm, Hu-14		6,401.00 USD
	5188-6557	Multiple Affinity Removal Column, 4.6 x 50 mm, Hu-14		4,002.00 USD

QTY	PART NUMBER	UNIT	DESCRIPTION	LIST PRICE	
	5185-5987	1 L	Buffer A - a proprietary solution for Multiple Affinity Removal LC Columns & Spin Catridges, 1L	$95.33	

Buffer A - a proprietary solution for Multiple Affinity Removal LC Columns & Spin Catridges, 1L

UNSPSC : 47101613

QTY	PART NUMBER	UNIT	DESCRIPTION	LIST PRICE	
	5185-5988	1 L	Buffer B - a proprietary buffer for Multiple Affinity Removal LC Columns & Spin Catridges, 1L	$130.00	

Buffer B - a proprietary buffer for Multiple Affinity Removal LC Columns & Spin Catridges, 1L

Cartridge Starter Kit

Enter the desired item number and quantity to obtain product availability and pricing.

ADD TO CART

QTY	PART NUMBER	DESCRIPTION	PRICE
	5188-5254	Mult Aff Rem Spin Cartridge Reagent Kit 1L Buffer A, 1L Buffer B, 2/25pk spin filters, 1/25pk spin conc., 1pk luer 1k adapters, 1pk luer 1k syr, 6/100pk 1.5ml microtubes, 1pk caps/plugs, 1pk tef ndls	$744.00

HSA/IgG

Enter the desired item number and quantity to obtain product availability and pricing.

ADD TO CART

QTY	PART NUMBER	DESCRIPTION	PRICE
	5188-8825	Multiple Affinity Removal System Spin Cartridge, Human Albumin/IgG, 0.45 mL	$646.00

Protein isoform-specific validation defines multiple chloride intracellular channel and tropomyosin isoforms as serological biomarkers of ovarian cancer

Hsin-Yao Tang[a], **Lynn A. Beer**[a], **Janos L. Tanyi**[b], **Rugang Zhang**[c], **Qin Liu**[a], and **David W. Speicher**[a,*]

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[c]Gene Expression and Regulation Program, The Wistar Institute, Philadelphia, PA, USA

Samples were depleted of 20 abundant serum proteins using a ProteoPrep20 Immunodepletion Column (Sigma-Aldrich)

- **ProteoPrep® 20 Plasma Immunodepletion LC Column**

Price and Availability

SKU-Pack Size	Availability	Price (EUR)	Quantity
PROT20LC-1EA	Estimated to ship on 01.06.15	22,200.00	0

Proteoprep 20 is an immunodepletion liquid chromatography (LC) column that removes 20 abundant interferring proteins from plasma or serum. Proteoprep 20 removes: albumin, IgG, transferrin, fibrinogen, IgA, α2- Marcroglobulin, IgM, α1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, A3 and B; α1- Acid Glycoprotein, ceruloplasmin, complement C4, C1q; IgD, prealbumin, and plasminogen.

The depleted, biologically significant remaining proteins can then be concentrated at least 20-fold.

The ProteoPrep20 LC Column can accommodate a plasma or serum sample of 100 microliters, and will deplete 100 samples or more. This effectively enables immunodepletion of 10,000 microliters of plasma or serum.

The ProteoPrep20 LC Column is designed for use with low or medium pressure liquid chromatography systems

Journal of proteome research

ART

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Combined Proteomic and Metabolomic Profiling of Serum Reveals Association of the Complement System with Obesity and Identifie Novel Markers of Body Fat Mass Changes

Andreas Oberbach,[†,‡] Matthias Blüher,[†,§] Henry Wirth,[∥,⊥] Holger Till,[‡] Peter Kovacs,[#] Yvonne Kullnic Nadine Schlichting,[†,‡] Janina M. Tomm,[∥] Ulrike Rolle-Kampczyk,[¶] Jayaseelan Murugaiyan,[○] Hans Bind Arne Dietrich,[▽] and Martin von Bergen*[,∥,¶]

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[‡]Department of Pediatric Surgery, University of Leipzig, Leipzig, Germany
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[∥]Department of Proteomics, Helmholtz Centre for Environmental Research, Leipzig, Germany
[⊥]Interdisciplinary Centre for Bioinformatics, University of Leipzig, Leipzig, Germany
[#]Interdisciplinary Centre for Clinical Research, University of Leipzig, Leipzig, Germany
[¶]Department of Metabolomics, Helmholtz Centre for Environmental Research, Leipzig, Germany
[▽]Department of Visceral, Thoracic, Vascular and Transplant Surgery, University of Leipzig, Leipzig, Germany
[○]Institute of Animal Hygiene and Environmental Health, Freie University Berlin, Philippstr. 13, 10115 Berlin, Germany

- High-Abundance Protein Depletion.

Twenty high-abundance proteins were depleted from plasma using the ProteoPrep 20 Plasma Immunodepletion Kit (Sigma Aldrich, Steinheim, Germany). Immunodepletion spin columns with 300µLof packed medium were used for depleting 100µL of diluted plasma (dilution 1:12.5 with equilibration buffer). Concentration of multiple depletions was carried out using Ultrafree-MC Microcentrifuge Filters (NMWL 5000 Da). Protein concentration of whole and depleted plasma was determined using the Bradford Reagent (Sigma Aldrich) with BSA as standard.

PROT20 SIGMA ProteoPrep® 20 Plasma Immunodepletion Kit

- The ProteoPrep 20 Plasma Immunodepletion Kit specifically removes 20 of the most abundant proteins from human plasma or serum in preparation for further proteomics analysis. This depletion enables deeper penetration into the plasma proteome whether you use one- or two-dimensional electrophoresis, single or multidimensional chromatography or go straight to mass spectrometry. This is based on a unique conjugation process using small recombinant immunoaffinity ligands and conventional antibodies that permits high density conjugation on the support. The ligand selection ensures optimal specificity. Typical depletions remove 97-98% of the total protein mass in human plasma or serum.

SKU-Pack Size	Availability	Price (EUR)	Quantity
PROT20-1KT	Estimated to ship on 23.06.15	4,705.00	0

ProteoPrep® 20 Plasma Immunodepletion Kit Single

SKU-Pack Size	Availability	Price (EUR)	Quantity
PROT20S-1KT	Only 6 left in stock (more on the way) - FROM	1,695.00	0

ProteomeLab™ IgY Protein Partitioning Kits

Formats

The format of which chemistry to use should be selected on the basis of:

- The volume of biological fluids needed to yield the target quantity of partitioned protein for subsequent analysis
- The sample throughput requirements of your lab

Spin Column (SC)

The IgY spin column format is intended for more analytical scale analyses and utilizes centrifugation as the force for affinity separation. The IgY SC spin column format is available in IgY-12 and IgY-R7, as well as individual partitioning solutions for HSA, BSA, RSA, total IgG, fibrinogen, transferrin, and HDL. As two spin columns are provided with each kit, you get double the throughput of other spin column solutions.

LC10 Column

The IgY LC10 column format is a high capacity chemistry utilizing IgY microbeads packed into a 10 mL column bed with liquid chromatography used as the force for affinity separation. The IgY-R7 LC10 column has a capacity of 100 µL mouse or 200 µL rat serum/plasma per LC column cycle. The IgY-12 column has a capacity of 250 µL human/primate serum or plasma.

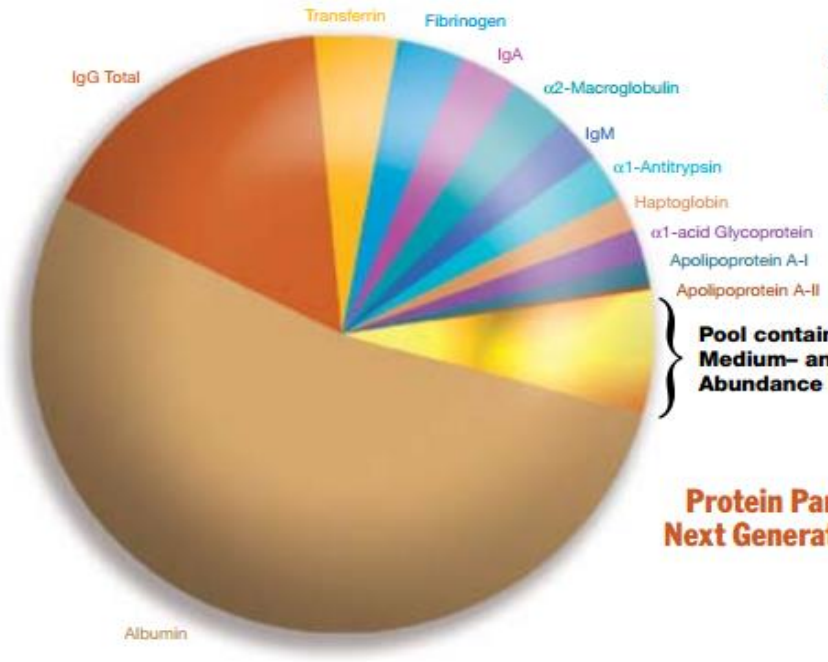
LC2 Column

The IgY LC2 column format is a moderate capacity chemistry utilizing IgY microbeads packed into a 2 mL column bed with liquid chromatography used as the force for affinity separation. This column has a capacity of 50 µL human/ primate serum or plasma per IgY-12 LC2 column cycle, while the IgY-R7 LC2 column kit has a capacity of 20 µL mouse or 40 µL rat serum/ plasma per LC column cycle.

High Capacity Format Yields 2 mg LAP/Cycle

By combining the capacity of the ProteomeLab IgY-12 LC10 column with fractionation, you are able to prepare the equivalent of 20 mg* (~250 µL) of plasma or serum proteins yielding 2 mg of low abundant proteins (LAP) for biomarker discovery per cycle. Five cycles of the IgY-12 LC10 affinity column enrich approximately 10 mg of protein for downstream analysis.

*Assuming plasma/serum protein concentration is 80 mg/mL



12 Proteins comprise up to 96 % of the protein mass in plasma.

Protein Partitioning – Next Generation Protein Depletion

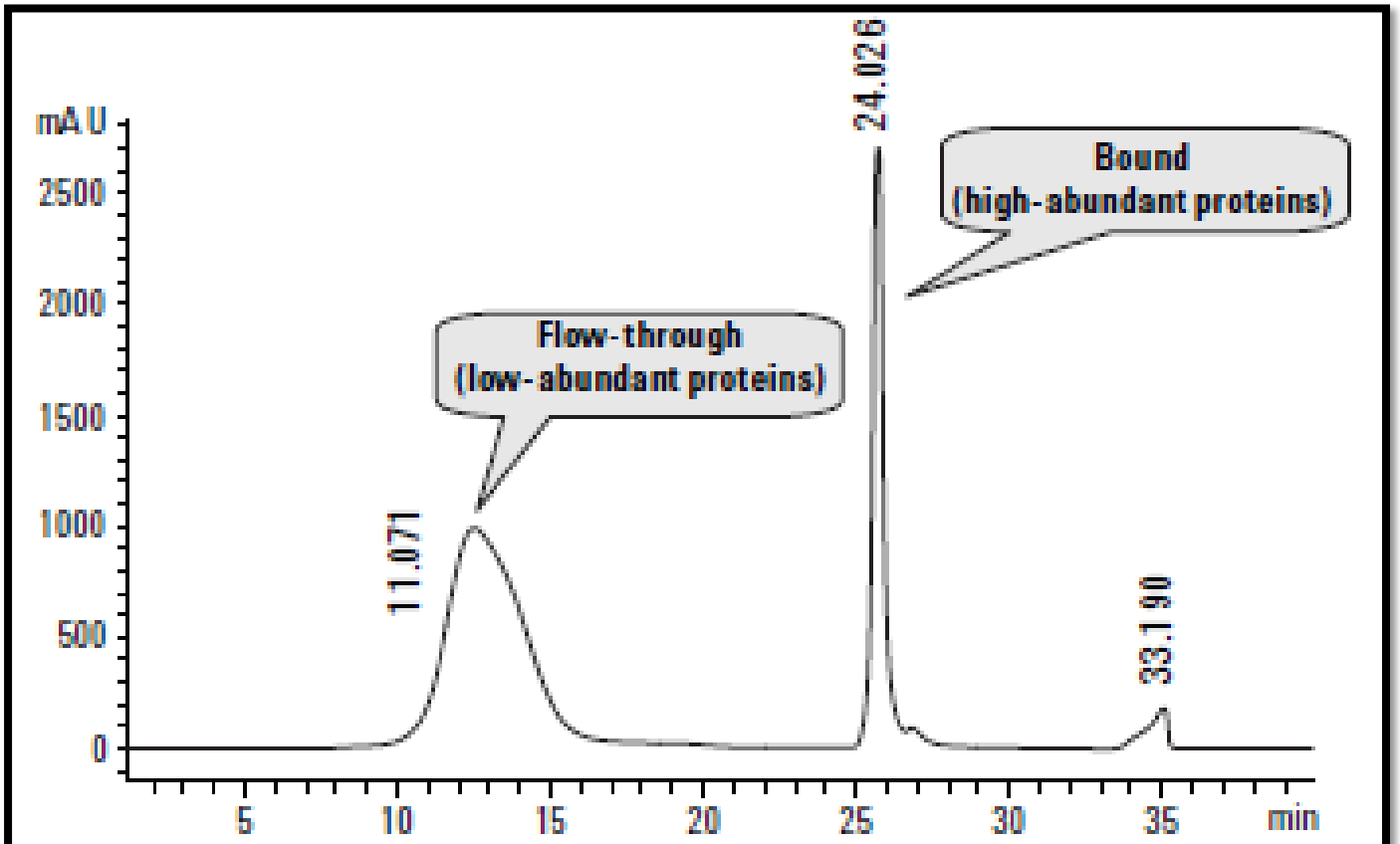


Figure 3 Representative chromatogram of 4.6 x 100 mm column.

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High abundant protein removal from rodent blood for biomarker discovery

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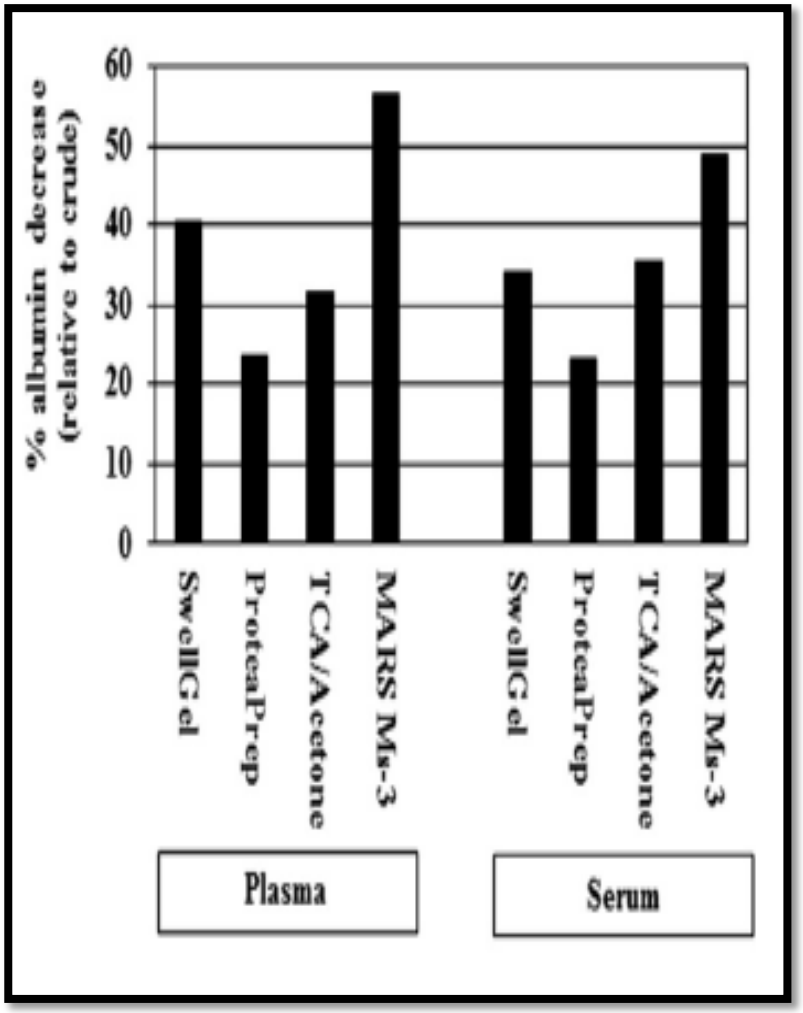
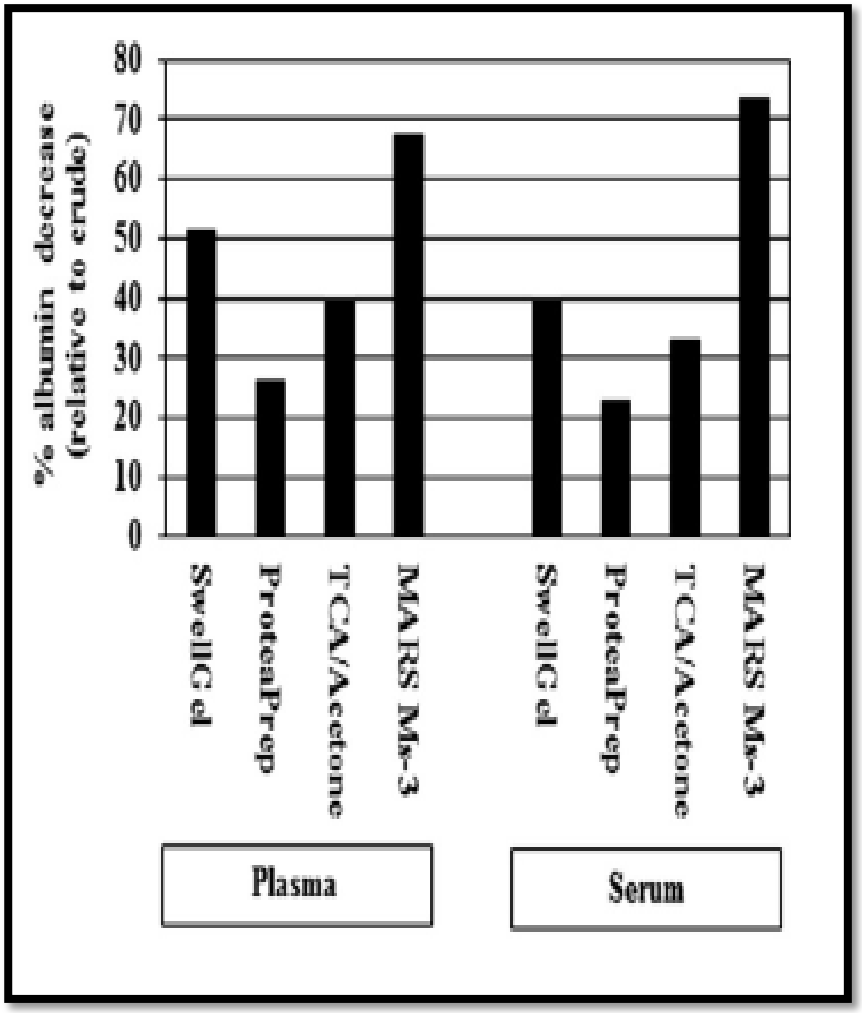
Unfortunately, high abundant proteins and complexity of the blood proteome present significant challenges for the discovery of protein biomarkers from blood. Animal models often enable the discovery of biomarkers that can later be translated to humans.

twenty-two proteins comprise over 90% of the total protein mass in human serum and albumin alone accounts for over %50



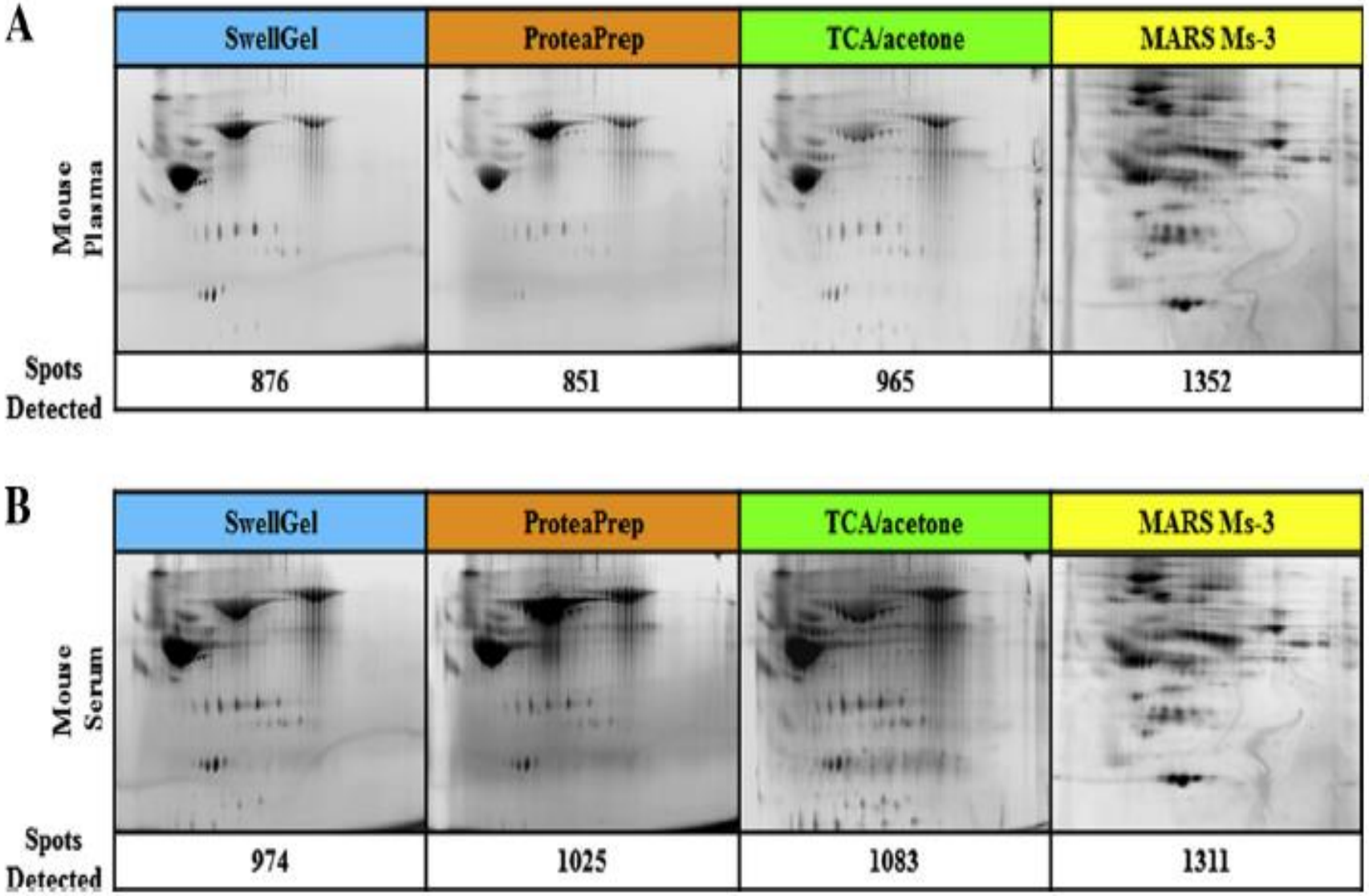


A

Species	Mouse							
Sample Type	Plasma				Serum			
Albumin Removal Method	SwellGel	Protea Prep	TCA/ acetone	MARS Ms-3	SwellGel	Protea Prep	TCA/ acetone	MARS Ms-3
Albumin Removed (SDS-PAGE)	51%	26%	39%	68%	39%	23%	33%	73%
Total 2-D Spots Detected	876	851	965	1352	974	1025	1083	1311

B

Species	Rat							
Sample Type	Plasma				Serum			
Albumin Removal Method	SwellGel	Protea Prep	TCA/ acetone	MARS Ms-3	SwellGel	Protea Prep	TCA/ acetone	MARS Ms-3
Albumin Removed (SDS-PAGE)	40%	24%	32%	56%	34%	23%	36%	49%
Total 2-D Spots Detected	1008	943	1157	1285	1069	1069	1155	1263


A
SwellGel
ProteaPrep
TCA/acetone
MARS Ms-3
Mouse Plasma
Spots Detected
876
851
965
1352
B
SwellGel
ProteaPrep
TCA/acetone
MARS Ms-3
Mouse Serum
Spots Detected
974
1025
1083
1311

Successful proteomic sample preparation from blood often requires high abundant protein removal. High abundant proteins have been shown to be responsible for concealing putative markers.

results clearly show that antibody-based affinity chromatography is the superior method for this approach.

antibody-based affinity chromatography removes the greatest percentage of albumin and results in the highest total number of lower abundant protein spots relative to any published work to date. We used 2-D DIGE