Calbiochem®
ProteoExtract™ Removal Kits

Enhancing Resolution of Low Abundance Proteins

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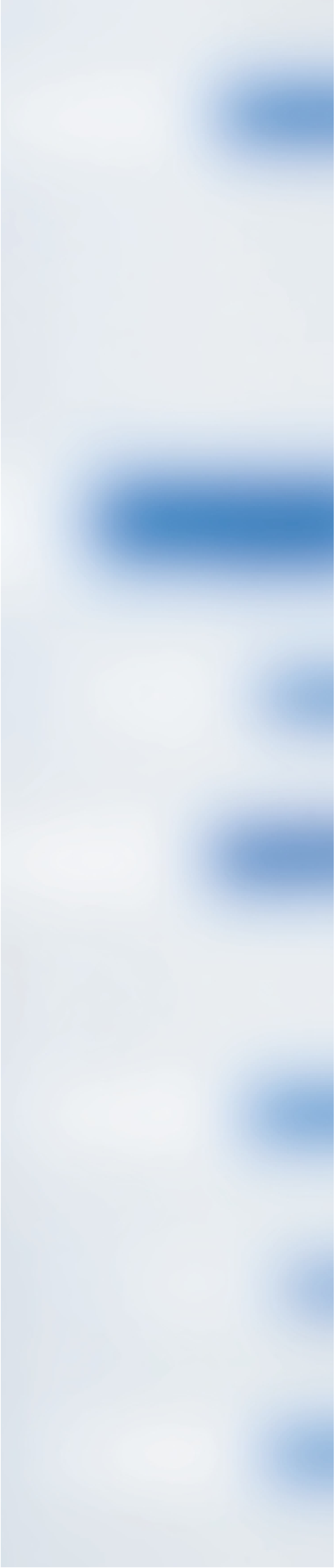
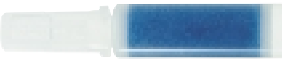
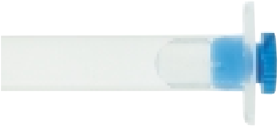




Enhancing Resolution of Low Abundance Proteins

The best sample sources for the identification of potential disease markers are serum or other body fluids as they present the largest version of the human proteome present in any sample. However, a major challenge for disease marker identification in human serum or plasma by proteome analysis is the extraordinary range of abundance of proteins present in the sample. Proteins in plasma differ in concentration by a factor of one billion. Serum albumin can constitute 55% of total serum proteins and IgG 10-25%. The presence of these proteins can obscure others and make the resolution of lower abundance proteins difficult. Removal of serum albumin and IgG eliminates approximately 75% of the total protein present in serum, allowing the visualization and analysis of the remaining proteins.



ProteoExtract™ Abundant Protein Removal Kits

The new ProteoExtract™ Abundant Protein Removal Kits provide highly specific and efficient depletion of either albumin alone or albumin and IgG from body fluids such as plasma or serum. Sample complexity is significantly reduced, enabling the detection of less abundant proteins. Depletion of albumin and IgG removes up to 75% of total serum proteins so that a 3-4 times more enriched sample can be loaded on 2DGE or liquid chromatography columns.

The ProteoExtract™ Abundant Protein Removal procedure is performed using pre-filled disposable gravity-flow columns, allowing the parallel processing of multiple samples. No centrifugation is required. Each kit contains columns and buffer sufficient for 12 extractions. Depleted serum samples are ready for downstream analysis by liquid chromatography or 1 or 2DGE. Two ProteoExtract™ Abundant Protein Removal Kit options are available: ProteoExtract™ Albumin Removal Kit for removal of albumin alone and ProteoExtract™ Albumin/IgG Removal Kit for simultaneous removal of albumin and IgG in one step.

Features and Benefits

- Efficient removal of albumin and IgG, enables the visualization of low abundance proteins
- Highly specific, exhibiting little to zero non-specific binding (not Cibacron based)
- Increased loading of enriched sample on 2DGE or LC
- Fast, 20 – 30 minute protocols
- Easy, column based procedure to process multiple samples in parallel

ProteoExtract™ Albumin and Albumin/IgG Removal Kit

The ProteoExtract™ Albumin Removal Kit is based on a new affinity resin which is highly specific for albumin. The Albumin/IgG Removal Kit uses a combination of the albumin specific resin and a unique immobilized protein A polymeric resin. ProteoExtract™ Removal Kits provide a binding capacity of 0.7 mg IgG and/or 2 mg albumin per column. Depletion of albumin and IgG from typical human serum samples is consistently higher than 80 % without binding significant amounts of other serum proteins. The remarkable selectivity provided by the resins and the optimized design of the columns result in background binding of less than 10 % to other serum proteins. Sample volumes from 20 - 60 µl can be processed without any loss of selectivity. ProteoExtract™ Abundant Protein Removal Kits have been optimized to bind human serum albumin and IgG, but will also deplete rabbit, rat or mouse samples effectively using the same protocol.

Each kit contains 12 disposable micro-columns pre-packed with either 300 µl of the albumin resin alone, ore 450 µl of the resin mix and a special binding buffer which promotes selective binding of albumin and IgG. The gravity-flow column format minimizes hands on time making it highly suitable for easy handling of multiple samples in parallel. The ProteoExtract™ Abundant Protein Removal Kits depletion procedure is convenient and straight forward: equilibrate, add the diluted sample, allow to pass by gravity-flow, wash the column, combine collected flow through and wash, then concentrate the proteins. Depleted samples are compatible with all downstream proteomics methods such as 1 and 2DGE, LC/MS or MALDI-TOF MS.

Efficient removal of albumin and IgG from human serum samples

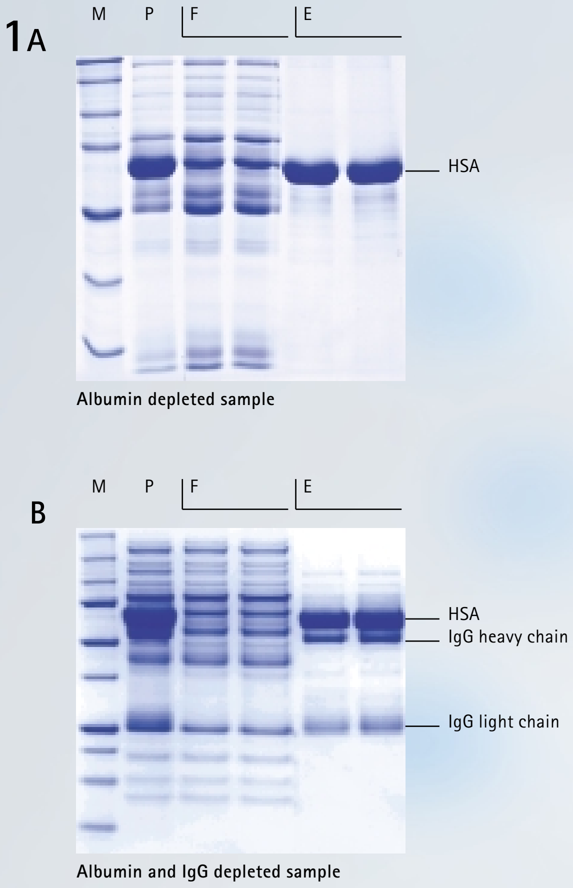


Fig. 1. 35 µl human plasma was processed with either **(A)** ProteoExtract™ Albumin Removal Kit, or **(B)** ProteoExtract™ Albumin/IgG Removal Kit. M: Marker proteins, P: Human plasma, F: Flow-through, E: Eluate fraction. 15 µg protein from each fraction was separated by SDS-PAGE and visualized by Coomassie™ staining. Densitometric analysis of stained bands demonstrated that more than 80 % of albumin **(A)** or albumin and IgG **(B)** are removed from the serum using the ProteoExtract™ Removal Kits.

Highly specific removal of albumin

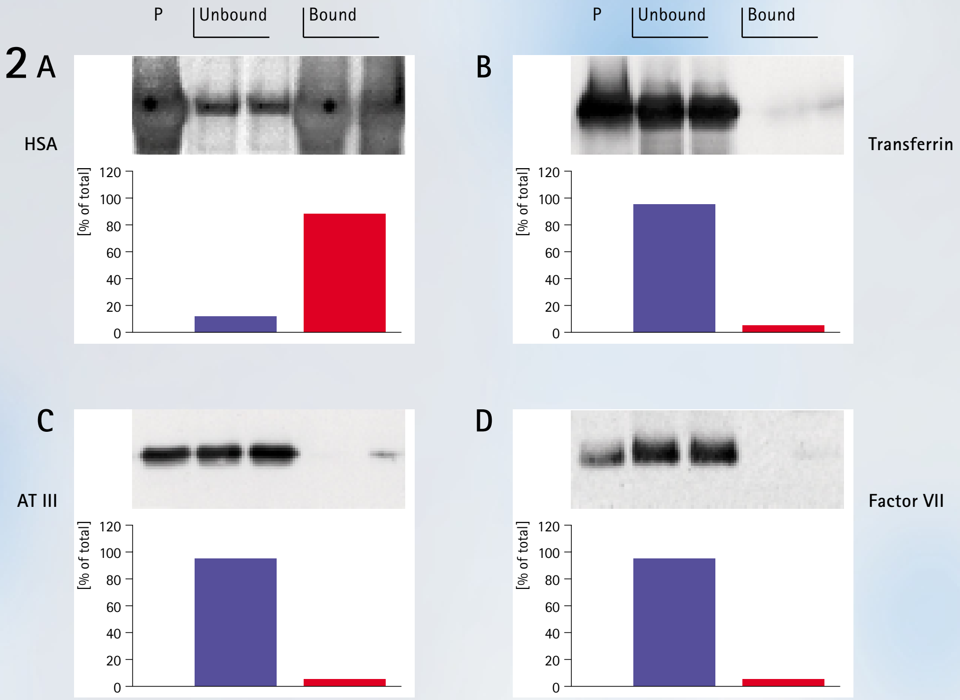
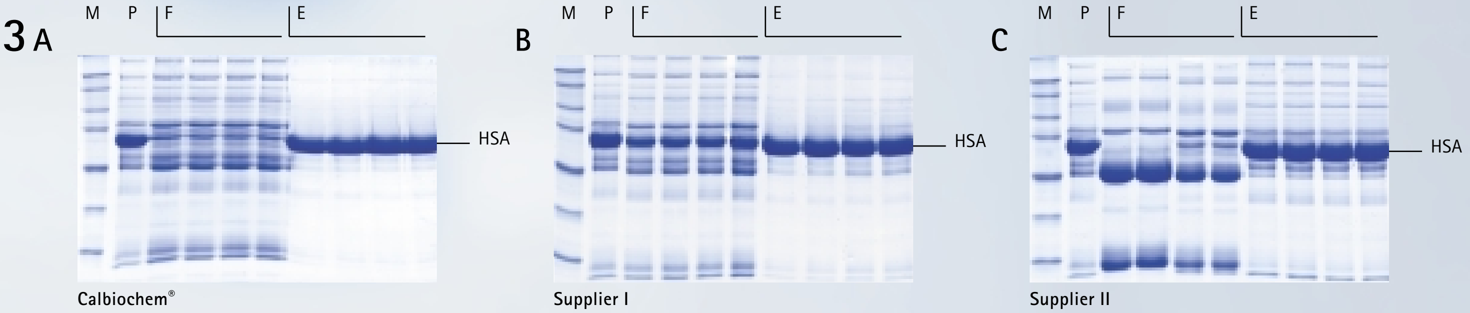


Fig. 2. 35 µl human plasma was processed with ProteoExtract™ Albumin Removal Kit.
A: 88 % of albumin is detected in the bound fraction demonstrating the high efficiency of the ProteoExtract™ Albumin Removal Kit.
B–D: More than 95 % of immunoreactivity against marker proteins is detected in the flow-through and less than 5 % of the three marker proteins remain in the bound fraction.
B: Transferrin, **C:** Antithrombin III and **D:** Factor VII

Lower background binding



Calbiochem®

Supplier I

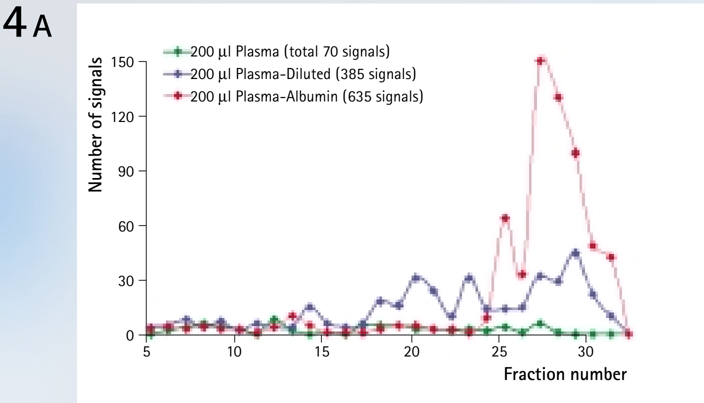
Supplier II

Fig. 3. Depletion of albumin from human plasma using the ProteoExtract™ Albumin Removal Kit is more specific and more efficient than with products from other suppliers. The same experiment was performed in parallel using albumin depletion products from Supplier I and Supplier II. M: Marker proteins, P: Human plasma, F: Flow-through, E: Eluate fraction. 15 µg protein of each fraction was separated by SDS-PAGE and visualized by Coomassie™ staining. **A:** The absence of other proteins than albumin in the eluate fraction demonstrates the low background binding using the ProteoExtract™ Removal Kit. High background binding of proteins and less efficient removal of albumin is seen using products from Supplier I **(B)** and Supplier II **(C)**.

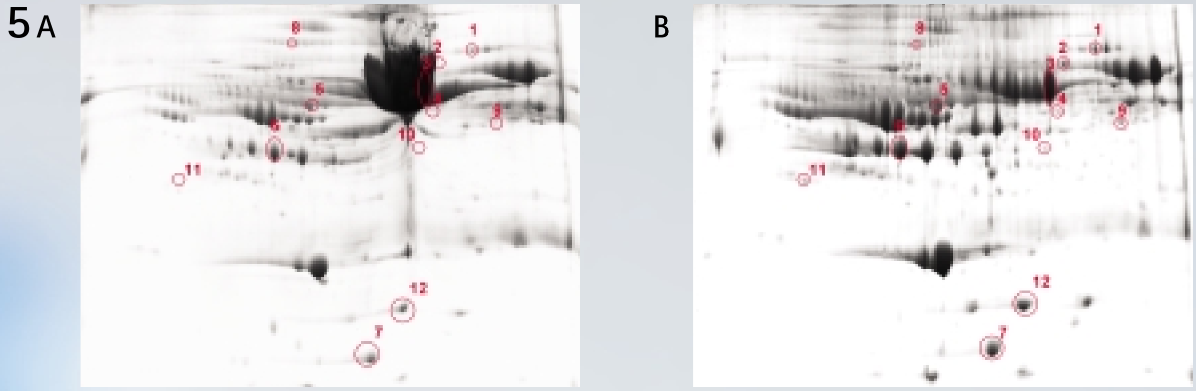
Improving Biomarker Discovery

The albumin and IgG depleted samples are compatible with all types of downstream proteomics methods such as classical 2DGE, LC/MS or MALDI-TOF MS. Depletion of abundant proteins from human plasma using the ProteoExtract™ Abundant Protein Removal Kits allows for the detection of a higher number of proteins and peptides for biomarker discovery, not only by 2DGE but also using liquid chromatography.

Detection of a higher number of peptides after depletion of albumin



Visualization of previously undetected proteins



Unprocessed human serum

Spot	Identity
1	complement Factor B Precursor
2	human serum albumin fragment
3	human serum albumin
4	human serum albumin
5	antithrombin III, chain L
6	haptoglobin chain beta
7	transthyretin
8	inter-alpha trypsin inhibitor - related protein precursor
9	human serum albumin precursor
10	non-identified
11	non-identified
12	haptoglobin chain alpha 2

Albumin and IgG depleted serum

Spot	Identity
1	complement Factor B Precursor
2	Gelsolin precursor
3	human serum albumin
4	transferrin n-terminal lobe
5	antithrombin III, chain L
6	haptoglobin chain beta
7	transthyretin
8	inter-alpha trypsin inhibitor - related protein precursor
9	transferrin fragment HUMTF12 NID
10	apolipoprotein L1 precursor
11	alpha-1-antitrypsin chain A
12	haptoglobin chain alpha 2

Fig. 5. 35 µl of human serum was either used directly **(A)** or subjected to albumin/IgG depletion **(B)** using the ProteoExtract™ Albumin/IgG Removal Kit. 1.5 mg of each fraction was precipitated, resolubilized in IEF buffer, subjected to 2DGE and visualized by Coomassie™ staining. Selected spots were excised from the gel, proteins were digested with trypsin and identified by peptide mapping using nano-LC/MS and the Mascot search algorithm. The identification of 5 additional proteins, e.g. spots 2, 4, 9, 10 and 11 in the depleted serum sample **(B)** demonstrates that removal of albumin and IgG allows the visualization and identification of additional proteins otherwise obscured by albumin and IgG.

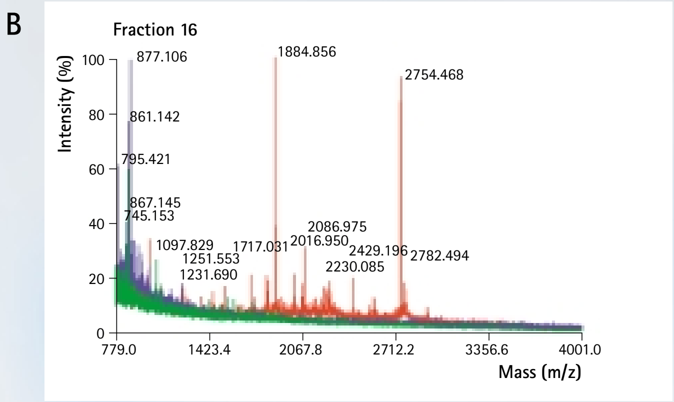


Fig. 4. Human plasma was subjected to albumin removal using the ProteoExtract™ Albumin Removal Kit followed by ion-exchange chromatography (IEC) and reversed phase clean-up prior to MALDI-TOF analysis. **A.** The red line demonstrates that a higher number of peptides was detected by MALDI-TOF MS when albumin was removed from plasma compared to sample preparation without albumin removal (635 signals compared to 385 -blue line- and 70 signals -green line-, respectively). **B.** After removal of albumin from plasma, the signal-to-noise ratio (examplified for fraction 16 of the above mentioned IEC) using MALDI-TOF MS is improved compared to the non-depleted samples.

Ordering Information

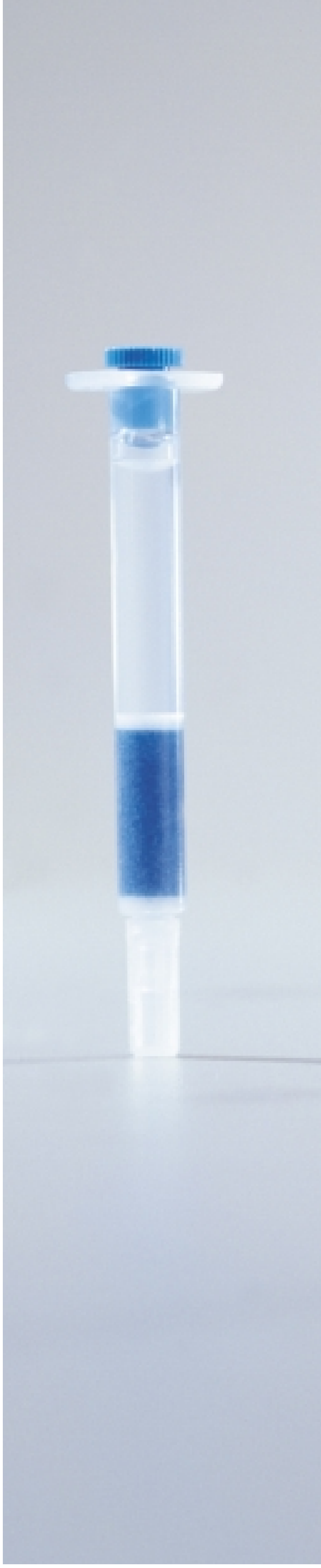
ProteoExtract™ Removal Kits

Product	Contents	Cat. No.*
ProteoExtract™ Abundant Protein Removal Kits		
ProteoExtract™ Albumin Removal Kit	Reagents to process 12 samples: 12 Columns, Binding Buffer	122640
ProteoExtract™ Albumin/IgG Removal Kit	Reagents to process 12 samples: 12 Columns, Binding Buffer	122642

Other ProteoExtract™ Kits

Product	Contents	Cat. No.*
ProteoExtract™ Subcellular		
Proteome Extraction Kit	Reagents to process 20 mammalian samples, yielding 4 subcellular fractions each	539790
ProteoExtract™ Complete Kits		
ProteoExtract™ Complete Bacterial Kit	Reagents to process 20 bacterial samples	539770
ProteoExtract™ Complete Yeast Kit	Reagents to process 20 yeast samples	539775
ProteoExtract™ Complete Mammalian Kit	Reagents to process 20 mammalian samples	539779
ProteoExtract™ Partial Kits		
ProteoExtract™ Partial Bacterial Kit	Reagents to process 20 bacterial samples, yielding 4 fractions each	539780
ProteoExtract™ Partial Yeast Kit	Reagents to process 20 yeast samples, yielding 3 fractions each	539785
ProteoExtract™ Partial Mammalian Kit	Reagents to process 20 mammalian samples, yielding 4 fractions each	539789

* Prices and availability are subject to change without notification.



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