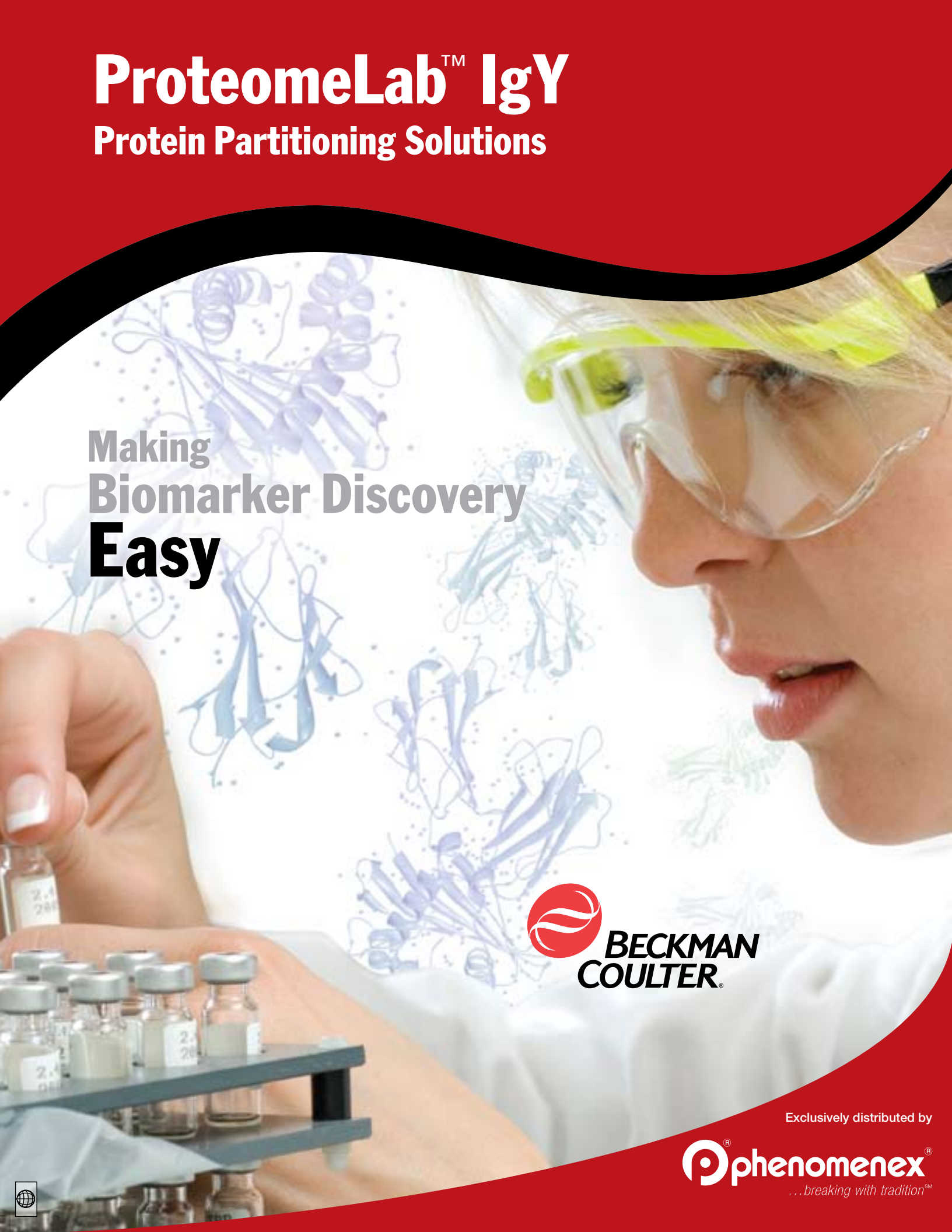
ProteomeLab™ IgY

Protein Partitioning Solutions



Making
Biomarker Discovery
Easy

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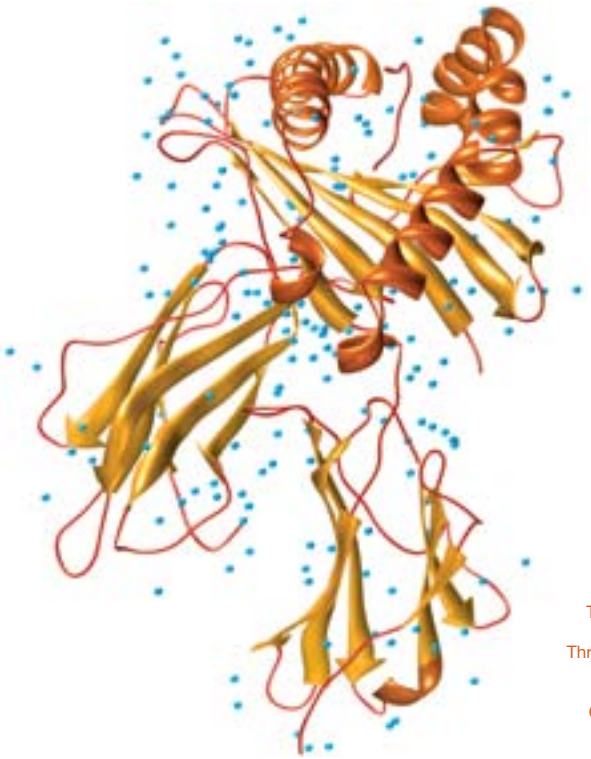
Biomarker Discovery— like finding a needle in a haystack

Finding the needle is much easier when there is less hay to sort through. Within biofluid proteomes such as plasma and serum, the "hay" is comprised of well-characterized proteins. Identifying the potential biomarkers in the presence of these highly abundant proteins has proven to be a serious challenge to current analytical techniques.



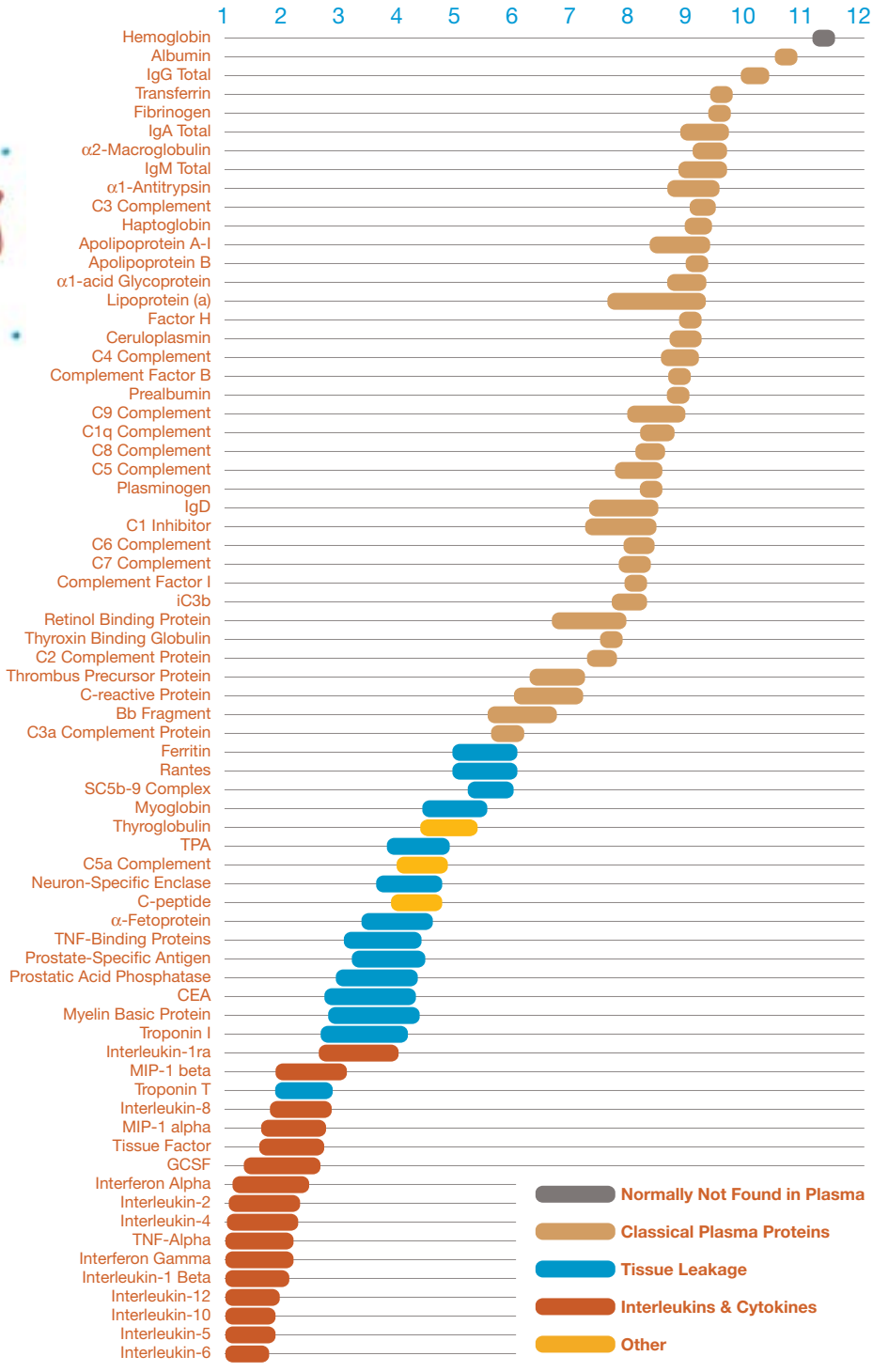
The Biomarker Discovery Challenge

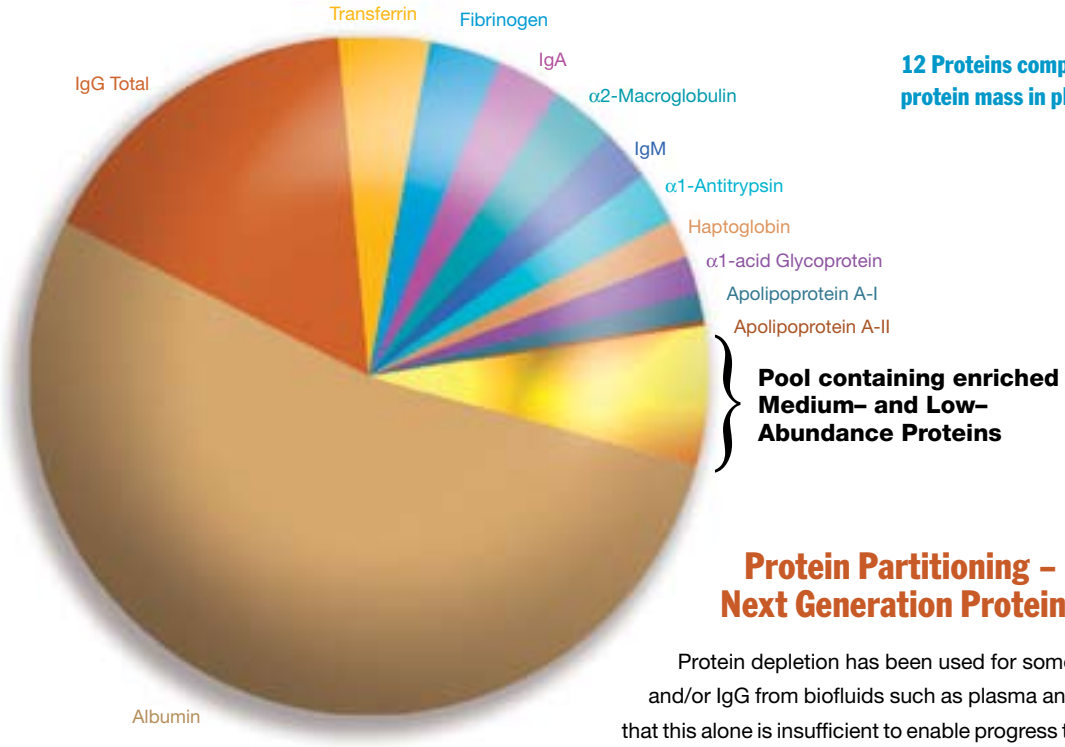
In serum and plasma, the dynamic range of proteins spans over 10 orders of magnitude, far greater than the measurement capability of current technologies. Additionally, potential biomarkers are entirely masked by the overwhelming abundance of relatively few proteins.

Typical Range Abundances Courtesy of Plasma Proteome Institute

The human plasma proteome: History, character, and diagnostic prospects. Anderson, N.L. and Anderson, N.G., Molecular and Cellular Proteomics, 1.11, 845-867 (2002).

Typical Protein Abundances in Human Plasma, Log10 Concentration in pg/ml





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

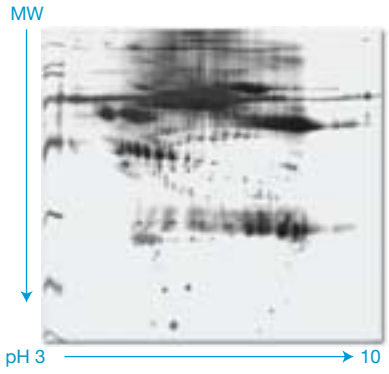
Protein Partitioning – Next Generation Protein Depletion

Protein depletion has been used for some years to remove most of the albumin and/or IgG from biofluids such as plasma and serum prior to analysis, but it is clear that this alone is insufficient to enable progress to be made in biomarker discovery. The presence of highly abundant proteins significantly complicates the discovery process by masking the presence and limiting the detection of low abundance species. ProteomeLab IgY partitioning addresses this issue by reversibly capturing the more abundant proteins from human biofluids such as plasma and serum, yielding an enriched pool of low abundance proteins for further study. The captured proteins can also be easily recovered for investigation if required - hence the term partitioning rather then depletion.

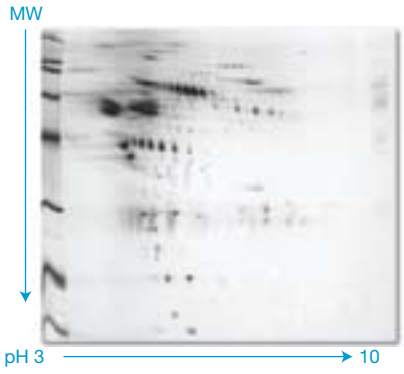
IgY-12 will selectively partition the 12 highly abundant proteins highlighted in the above illustration. The partitioned fractions can be taken to the next stage of the discovery process, such as multi-dimensional fractionation or profiling using 2D PAGE.

Unmask Potential Biomarkers

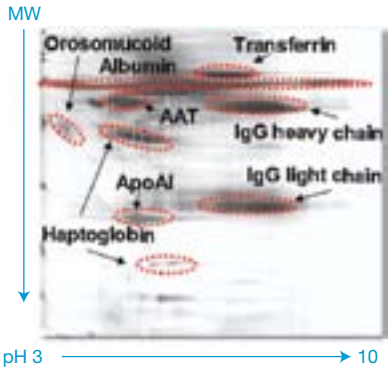
Analysis of partitioned human serum by 2D PAGE illustrates the highly abundant proteins that can be separated and the less abundant proteins in the enriched sample that then become apparent.



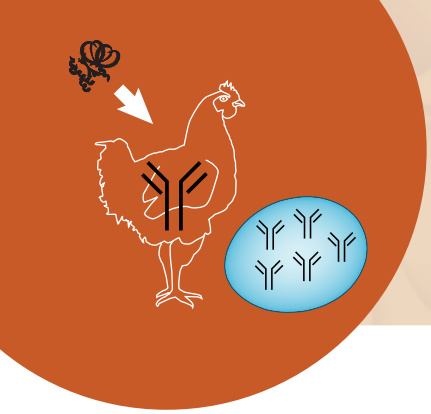
Unfractionated Human Serum

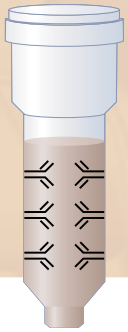


Enriched Low Abundance Proteins



12 Bound Abundant Proteins





Why IgY?

IgY antibodies are produced by injecting an avian species (chicken) with highly purified mammalian protein antigens. IgY chemistries offer broader antigen-binding host range and cleaner capture than IgG capture methods because of the evolutionary distance between chickens and mammals.

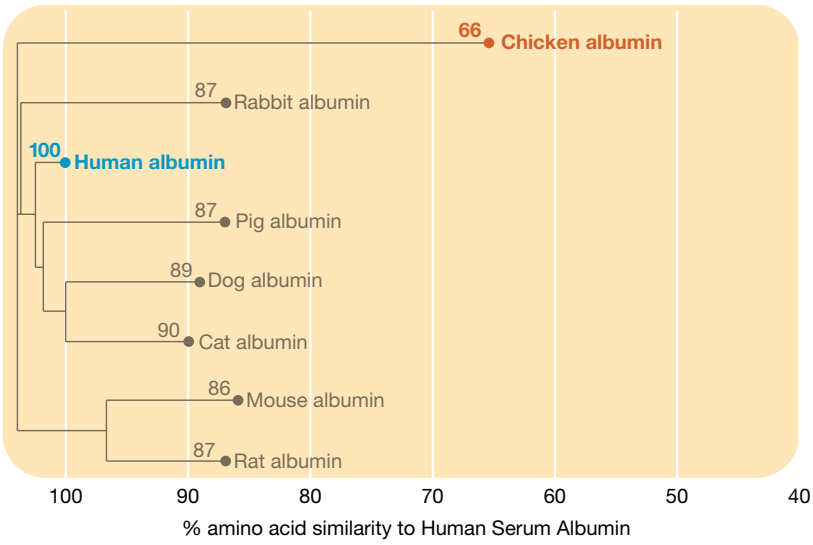
Broader Antigen-binding Host Range

Due to the phylogenetic difference, mammalian proteins are often more immunogenic in birds than in mammals,* hence the broader antigen-binding host range.

ProteomeLab IgY Chemistry effectively leverages this well-documented evolutionary advantage.

*Gassman, M., Thoemmes, P., Weiser, T., and Huebscher, U. (1990) *Efficient production of chicken egg yolk antibodies against a conserved mammalian protein*. FASEB J. 4, 2528-2532

Multiple Protein Alignment using Clustal X and Tree View



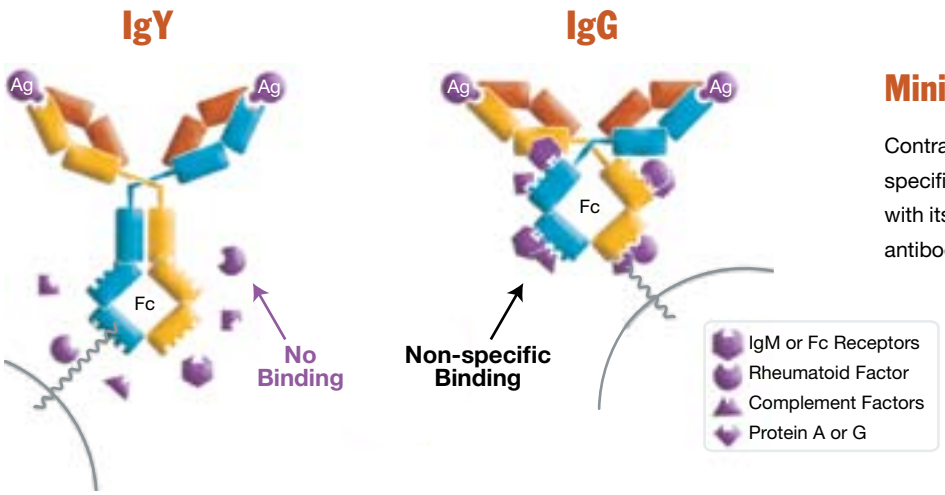
Cleaner Capture

As the polyclonal IgYs are avian, the Fc region of the antibody does not bind mammalian complement factors, Rheumatoid Factor, IgM, Fc receptor and Protein A or G, a known issue related to capture with mammalian antibodies. Coupled with high specificity across multiple epitopes, you get very specific binding across species while achieving very clean capture with little background. The SDS-PAGE highlights whole serum run in lane #1, serum with just albumin partitioned out by ProteomeLab IgY-HSA SC chemistry in lane #2, and the isolated/ partitioned albumin captured cleanly in lane #3. The partitioning efficiency of the reagents from human plasma is highlighted in the adjoining table. To further improve capture, both primate optimized 12-plex, and rodent optimized 7-plex affinity solutions are available.

Partitioning Efficiency >100 Cycles on IgY-12 LC10 Column

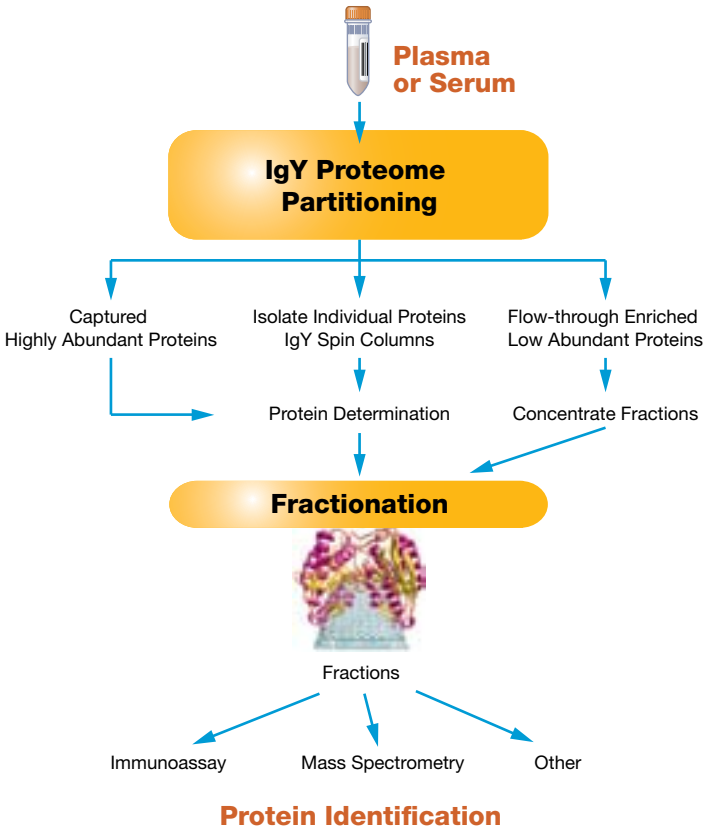
ELISA analytical method

Protein	Acceptance Criteria	Typical QC Results
Human Serum Albumin	>99 %	99.60 %
IgG Total	>99 %	99.11 %
Transferrin	>99 %	99.12 %
IgA	>95 %	99.00 %
IgM	>90 %	99.04 %
Apo A-I	>95 %	99.19 %
Apo A-II	>95 %	99.19 %
α1-Acid Glycoprotein	>90 %	99.07 %
α2-Macroglobulin	>90 %	94.35 %
α1-Antitrypsin	>95 %	99.73 %
Haptoglobin	>95 %	99.33 %
Fibrinogen	>90 %	96.89 %



Minimal Non-specific Binding

Contrary to IgG, IgY does not bind in a non-specific manner to the proteins listed. Combined with its broader host range, this is why IgYs are the antibodies of choice for proteome partitioning.



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

Discarding Part of the Proteome—No Longer a Concern

Whether you are fractionating or profiling biofluid proteomes, the presence of highly abundant proteins will mask the identity of many of the medium to lower abundance species. Now you can greatly enhance the detectability and identification of less abundant proteins through the selective partitioning of many of the highly abundant species. This process serves to both enrich the medium to low abundance proteins, while removing the deleterious masking effects generated by the overlapping peptide mass fingerprints from highly abundant proteins. In parallel, the captured highly abundant proteins are collected to allow for further analysis, as these proteins may also include a wealth of information. Either way, all protein fractions are preserved to allow you to monitor all aspects of the proteome.

Why partitioning?

Rather than employing a depletion strategy, proteome partitioning focuses on both the affinity capture of highly abundant proteins as well as the enrichment of the flow-through material.

Formats and Chemistries

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)

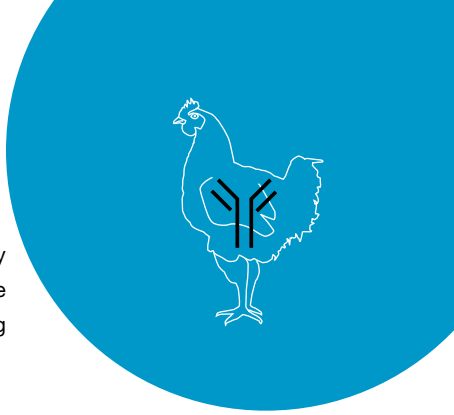
The IgY spin column format is intended for more analytical scale analyses and utilizes centrifugation as the force for affinity separation. The IgY SC spin column format is available in IgY-12 and IgY-R7, as well as individual partitioning solutions for HSA, BSA, RSA, total IgG, fibrinogen, transferrin, 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.

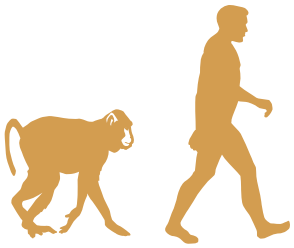
LC2 Column

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.



ProteomeLab IgY Chemistries

The ProteomeLab IgY proteome partitioning chemistries are based on affinity columns using avian (IgY) antigen interactions and optimized buffers for sample loading, washing, eluting and regenerating. The selective immunoaffinity partitioning provides an enriched pool of lower abundant proteins.



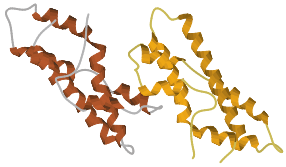
IgY-12

The ProteomeLab IgY-12 proteome partitioning chemistries are specifically designed to remove twelve highly abundant proteins from human/primate biological fluids such as serum, plasma, and cerebral spinal fluid (CSF). This technology enables removal of albumin, total IgG, α1-antitrypsin, IgA, IgM, transferrin, haptoglobin, α1-acid glycoprotein (orosmucoid), α2-macroglobulin, HDL (apolipoproteins A-I & A-II) and fibrinogen in a single step.

IgY-R7

The ProteomeLab IgY-R7 proteome partitioning chemistries are specifically designed to remove seven highly abundant proteins from rodent biological fluids such as serum, plasma, and cerebral spinal fluid (CSF). This technology enables removal of albumin, IgG, α1-antitrypsin, IgM, transferrin, haptoglobin and fibrinogen, in a single step.

In both cases, the targeted highly abundant proteins are simultaneously partitioned by the immobilized specific IgYs when complex biological samples are passed through the column. Selective immunoaffinity partitioning provides an enriched pool of lower abundant proteins for downstream proteomic analysis.



IgY Single-Component

The ProteomeLab IgY Single-Component proteome partitioning kits specifically remove any of the highly abundant individual proteins in a single step. Single component kits available include HSA, BSA, RSA, fibrinogen, HDL, IgG-Fc, and transferrin.

Ordering Information

Each kit includes: Two 1.2 mL spin columns (SC kits) or 6.4 x 63 mm column (LC2 kits) or 12.7 x 79 mm column (LC10 kits), dilution buffer, stripping buffer, neutralization buffer, spin filters (LC2 & LC10 kits), collection tubes (SC kits), and manual

Part No.	Description	Unit	Price
IgY-12 Chemistry			
CM0-8242	ProteomeLab™ IgY-12 LC10 Proteome Partitioning Kit	ea	
CM0-8243	ProteomeLab™ IgY-12 LC2 Proteome Partitioning Kit	ea	
CD0-8246	ProteomeLab™ IgY-12 SC Proteome Partitioning Kit	ea	
IgY-R7 Chemistry			
CM0-8244	ProteomeLab™ IgY-R7 LC10 Proteome Partitioning Kit	ea	
CM0-8245	ProteomeLab™ IgY-R7 LC2 Proteome Partitioning Kit	ea	
CD0-8247	ProteomeLab™ IgY-R7 SC Proteome Partitioning Kit	ea	
IgY Single Antibody Solutions			
CD0-8248	ProteomeLab™ IgY-HSA SC Proteome Partitioning Kit	ea	
CD0-8249	ProteomeLab™ IgY-BSA SC Proteome Partitioning Kit	ea	
CD0-8250	ProteomeLab™ IgY-RSA SC Proteome Partitioning Kit	ea	
CD0-8251	ProteomeLab™ IgY-IgG-Fc SC Proteome Partitioning Kit	ea	
CD0-8252	ProteomeLab™ IgY-Transferrin SC Proteome Partitioning Kit	ea	
CD0-8253	ProteomeLab™ IgY-Fibrinogen SC Proteome Partitioning Kit	ea	
CD0-8254	ProteomeLab™ IgY-HDL SC Proteome Partitioning Kit	ea	

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