NRicher™

A Low Abundance Proteome Enrichment Platform With Seamless Integration of On-Bead Digestion

Yes, we have been developing chemically derived beads with differential binding for sub-proteome separations, for over 10 years. Now we can increase the low abundance proteome coverage by using a series of differentiated beads into one advantaged platform, called NRicher™.

Introduction

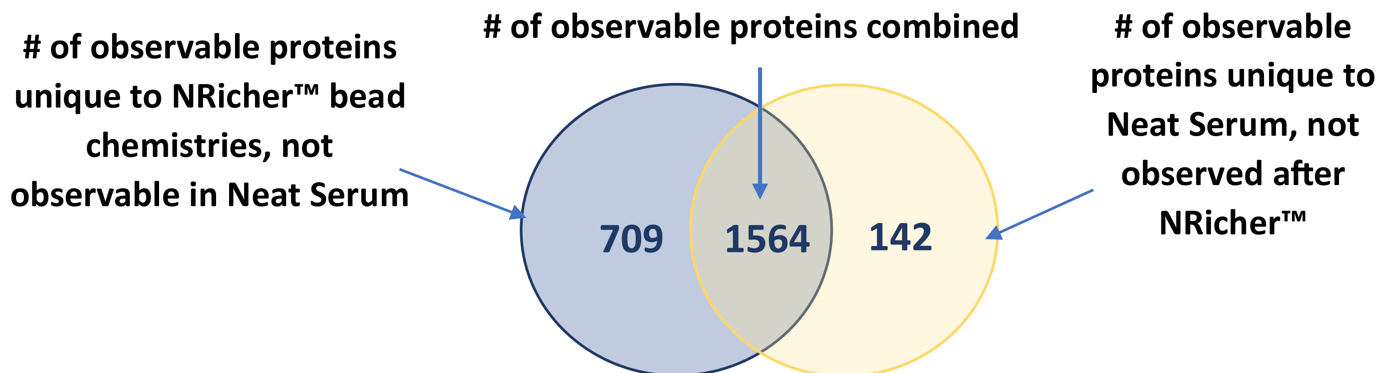
There has been a long-standing need for low-abundance proteome enrichment to overcome the dynamic range associated with highly abundant proteins. Recently, the introduction of magnetic nano-particles has presented a potential solution to interrogate the proteome using separations based on physicochemical properties, with formation of unique sub-proteome-bound coronas on non-porous beads. This approach, obviates the use of immuno-affinity, but necessitates an instrument-based workflow and the need to perform LC-MS analysis on each individual bead-derived sub-proteome. We herein present **NRicher™**, a porous bead-based platform as a much simpler and more versatile alternative. Fit-for-purpose products are available for both quantitative/ targeted (Complement, Immunoglobulins, Apolipoproteins), along with discovery/coverage applications, as described in the next sections.

The NRicher™ Advantage:

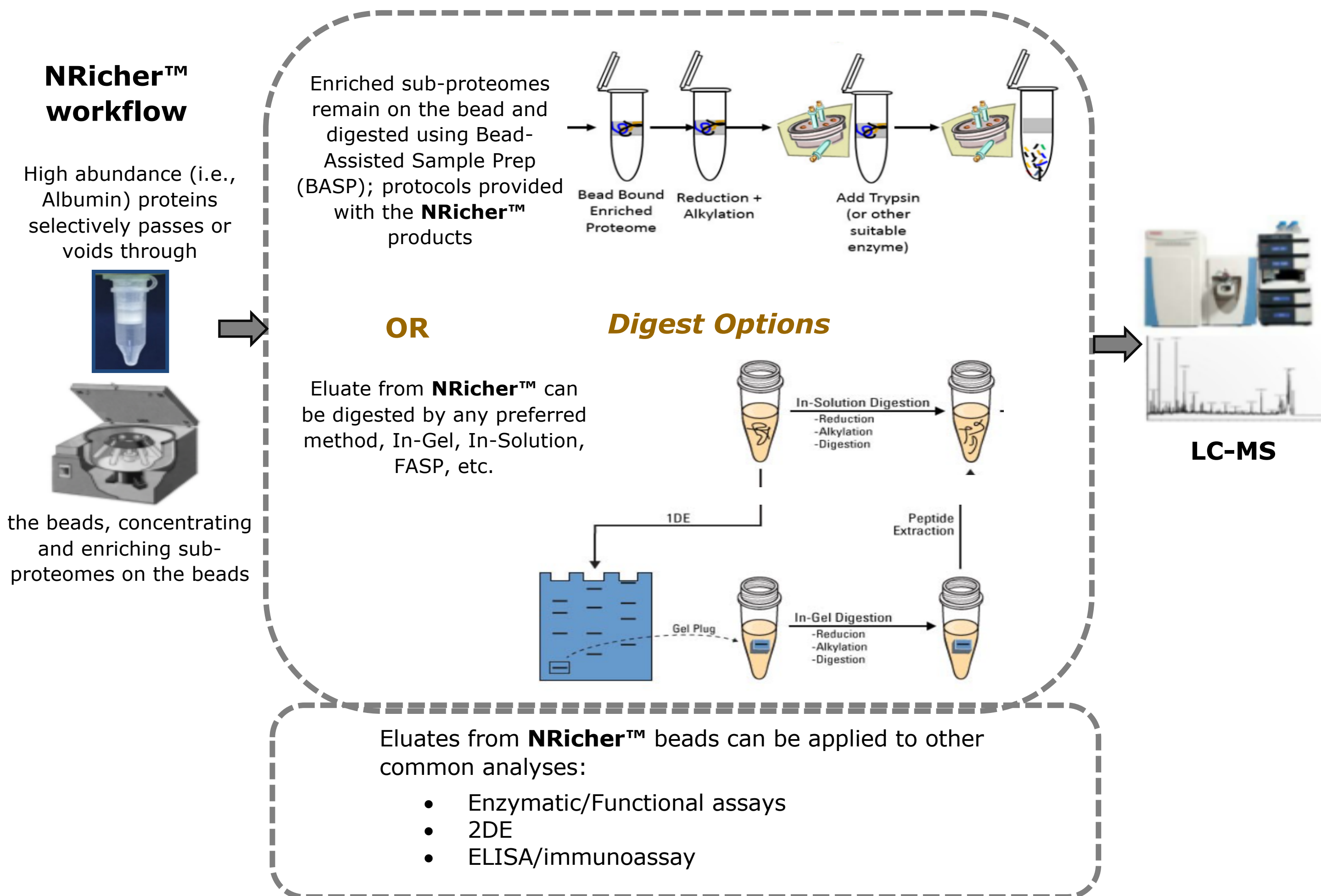
- **Consumable chemically derived NuGel™ beads, species agnostic as they are not derived from antibodies**
- **Does not require any specialized instruments, just a standard microfuge**
- **Use of bead cocktails allows for one, rather than multiple LC-MS analyses**
- **Functionally active sub-proteomes after separations, for any orthogonal functional, enzymatic, or immunoassay analysis**

At its foundation are porous silica-based NuGel™ beads, derivatized to produce surface features with different multi-dimensional separation characteristics. We and others have been reporting on such beads with differential binding properties (i.e., ionic, hydrophobic, hydrogen bonding, aromatic, polymeric) for sub-proteome separations, for over 10 years in both LC-MS and functional proteome analysis[4,5]. Two commercial enrichment products based on singular bead surface features have been derived, notably **AlbuVoid™** and **HemoVoid™**. These have been successfully employed in a variety of proteomic applications, reference examples provided on page 10.

Nevertheless, an adaptability to bead cocktails, even with seemingly incompatible surface features is an important distinction of porous, over non-porous beads. We now report on increasing the low abundance proteome coverage by using a series of differentiated beads into one platform, hereinafter called **NRicher™**. Derivative enrichment products dedicated to specific application and uses are described in the sections to follow.



Results reported here, are based on processing 100 µl of pooled normal human serum, on each **NRicher™** bead, and digested using BSG developed on-bead digest protocols. Neat samples were digested in-gel. The samples were analyzed at the Rutgers Center for Integrative Proteomics. Briefly, DIA (Data independent acquisition) workflow was used to analyze the eluted peptides. MS scan range were set at 400-1200, resolution 12,000 with AGC set at 3E6 and ion time set as auto. 8 m/z window were set to sequentially isolate (AGC 4E5 and ion time set at auto) and fragment the ions in C-trap with relative collision energy of 30. The MSMS were recorded with Resolution of 30,000. Raw data were analyzed with predicted library from Uniprot reference proteome for library-free search using DIA NN 1.8.1 with recommended setting[6].



The NRicher™ Workflow. Each **NRicher™** bead is processed the same, using buffers and spin-filters provided with the kits. The beads are supplied as a dry powder, weighed and dispensed into the top of a spin-filter, and follows a bind/wash protocol using a standard microfuge to separate the buffer solutions from the beads. Once the **NRicher™**-derived sub-proteome (different for each application) is bound to the beads, a variety of options are available to the user. Using protocols developed from previous products, in a process called Bead-Assisted Sample Prep (BASP™), reduction, alkylation and digestion is performed on the bead-bound proteome, without the use of detergents, seamlessly integrating to LC-MS analysis[1-3]. If users prefer other digest/denaturing conditions, the derived sub-proteome is easily eluted with moderately alkaline buffers, and can be adapted to common digest methods including In-Gel, In-Solution, FASP, etc. Eluates from **NRicher™** beads can also be used to improve signal to noise for other common analyses, such as enzymatic/functional assays, 2DE, or ELISA/immunoassay.

NRicher™ Products

NRicher™ Apo

Enrichment of Apolipoproteins

NRicher™ Ig

Enrichment of all isotypes and subclasses of Immunoglobulins

NRicher™ C

Enrichment of the Complement-related sub-proteome

NRicher™ MX

General Enrichment for any sample type

NRicher™ Apo

Enrichment of Apolipoproteins

Apolipoproteins play a key role in atherosclerotic processes. Apo E variants are under investigation in neurological disorders, and in cancer there is evidence for modulating apolipoprotein expression. Thus, profiles of circulating apolipoproteins hold promise as biomarkers for the prediction of cardiovascular disease (CVD) and other precision medicine applications. However, clinical immunoassays are not available for most apolipoproteins, and variants require special consideration. For example, the size polymorphism of Lp(a) necessitates a need for isoform-agnostic measurement. Thus, advances in the productivity and multiplexing capacity of LC-MS/MS, offer the potential for personalized profiling by simultaneous quantification of multiple apolipoproteins and their associated variants. The **NRicher™ Apo** product is dedicated to Apolipoproteins to help in these investigations. Another BSG product - **Cleanascite™** binds to only lipid-bound proteins, and can be used to investigate the lipid-bound vs. unbound profile. This may provide additional granularity to CVD risk assessment.

	Bead Enrichment Factor Relative To Neat	Bead Enrichment Factor Relative To Albumin
APOA1	30	349
APOB	3	35
APOA2	441	5062
APOA4	49	561
APOE	11	123
APOC1	741	8504
APOC3	40	458
APOH	3	31
APOC2	154	1768
APOD	5	58
APOL1	4	47
APOF	91	1040
APOM	4	48
APOC4	∞	∞
APOL3	∞	∞
APOA5	470	5394
LPA (apo(a))	1	13
Total All Apo	14	349

Bead Enrichment Factor Relative to Neat = (% of Gene Specific Signal relative to Total Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Total Signal from Neat)

Bead Enrichment Factor Relative to Albumin = (% of Gene Specific Signal relative to Albumin Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Albumin Signal from Neat)

∞ Indicates observed on bead, but not in neat

Product	Size	Total serum/plasma samples processed	Item No.
NRicher™ Apo	10 Preps	10 x (25-50) µl samples	NAPO-10
NRicher™ Apo	50 Preps	50 x (25-50) µl samples	NAPO-50

NRicher™ Ig

Enrichment of all isotypes and subclasses of Immunoglobulins

A comprehensive analysis of the humoral immune response (the immunome) has potential to greatly impact research across numerous fields. For example, serum autoantibodies against tumor-associated antigens have recently emerged as early stage biomarkers for different types of cancers.

Most autoantibody profiling work has been based on the reactivity of unbound antibodies towards antigens produced by a variety of strategies (i.e., cDNA libraries, phage display).

An alternative approach is based on the identification of Ig-bound antigens using Liquid Chromatography coupled to Mass Spectrometry (LC-MS). Such determination of antigens complexed with antibodies at a proteome scale is critical to understanding adaptive responses in the context of infection, autoimmunity, and cancer.

Human serum immunoglobulins comprise several classes IgG, IgA, IgM, IgD & IgE. Immunoglobulin G (IgG) is the predominant human immunoglobulin class in plasma and comprises four subclasses; ~60% are IgG_1, followed by ~30% IgG_2, ~7% IgG_3 and ~3% IgG_4. To date, most of the circulating antibody complex research has been focused on IgG as the efficiency of recovering a representative pool of IgG antibodies is well established. Generally for human serum/plasma, Protein A binds with high affinity to IgG_1, IgG_2, and IgG_4, but poorly to IgG_3.

	Bead Enrichment Factor Relative To Neat	Bead Enrichment Factor Relative To Albumin
IGHG1	1.7	12
IGHA1	6.1	44
IGKC	2.4	17
IGHG3	2.8	20
IGLC3	3.0	21
IGHG2	1.7	12
IGHG4	2.2	16
IGHM	1.3	9
IGHA2	4.1	29
IGHD	4.5	32
IGHE	4.0	29
Total All Ig	2.3	16

Bead Enrichment Factor Relative to Neat = (% of Gene Specific Signal relative to Total Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Total Signal from Neat)

Bead Enrichment Factor Relative to Albumin = (% of Gene Specific Signal relative to Albumin Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Albumin Signal from Neat)

Among the four IgG subtypes in mice, protein A has the weakest affinity for IgG_1 while Protein G has affinity for all four IgG subclasses. Neither Protein A or G bind particularly well towards IgA, IgM, IgD or IgE.

Nevertheless, the ability to enrich circulating immune complexes from sera or plasma from both animals and humans with high yield and without selective loss of isotypes or subclasses can provide more comprehensive profiles. **NRicher™ Ig** can provide such enrichment for all immunome profiling methods. For antigen reactivity profiling, elution conditions are mild (pH 9-10), and preserve functionality. For antigen identification, bound proteins can be digested on-bead, with seamless integration to LC-MS analysis.

Product	Size	Total serum/plasma samples processed	Item No.
NRicher™ Ig	10 Preps	10 x (25-50) µl samples	NIMM-10
NRicher™ Ig	50 Preps	50 x (25-50) µl samples	NIMM-50

NRicher™ C

Enrichment of Complement cascade related proteins

The complement cascade is a major component of the immune system that provides powerful host surveillance and protection from invading microbes. It also exerts an important influence on the adaptive immune response by acting synergistically with antibodies as well as promoting B- and T-cell stimulation. The intracellularly active complement system—the complosome, has been shown to play a critical function in regulating T cell responses, cell physiology (such as metabolism), and inflammatory disease processes. Complement dysregulation is implicated in chronic diseases such as age-related macular degeneration, paroxysmal nocturnal hemoglobinuria (PNH), along with neurologic, cardiovascular and oncology diseases. In acute inflammatory conditions, hyperactive complement may predispose individuals to adverse outcomes, as suspected in hospitalized Covid-19 patients.

The complement system consists of over 50 circulating and membrane proteins, comprising about 5% of the total protein mass in plasma. Most complement proteins circulate in blood as inactive precursors (zymogens), that when triggered, become activated through proteolytic cascades. Although textbooks describe three activation pathways (classical, lectin & alternative), leading to the protein aggregate C3-Convertase, Complement is also interconnected with Coagulation to eliminate damaged tissues; activating platelets and contributing to hemostasis in response to injury. Conversely, complement can be activated directly from proteolytic enzymes derived from coagulation (i.e., Thrombin, Kallikrein).

As a result of complement activation, many outcomes are produced including: opsonization of pathogens or damaged-self cells to enhance phagocytosis; production of anaphylatoxins C3a & C5a; recruitment of leukocytes to the inflammatory site; and the terminating end of the cascade – assembly of the membrane attack complex (MAC) on the cell surface.

The terminating end of the complement cascade is derived from the C3 Convertase proteolytic product - C5b, which engages the sequential recruitment of C6, C7, C8, and C9, assembling the membrane attack complex (MAC). Also known as the “terminal complement complex”, it results from the coordination of C5b-7 insertion in the membrane, which then captures C8, inducing polymerization of a C9 ring – to as many as 18, C9’s per pore. Terminal MACs perforate the cell membrane of the invading pathogen or target cell, and when a sufficient number of MAC pores form, the cell dies by osmotic lysis. Sub-lytic doses of MAC however, induce dramatically different effects than lytic doses, including adherence, aggregation, chemotaxis, cell division, and extracellular vesicle release.

In such a critical juncture, the complement system must maintain a delicate balance between activation and inhibition to allow activation when necessary to counteract infectious agents or modified self/host tissues, while concurrently protecting healthy self/host tissue. This protection is achieved systemically through the concerted action of regulators and inhibitors ensuring cell and tissue integrity essential for normal and healthy well-being. Notwithstanding such importance in disease and well-being, there are limited biomarkers to help in clinical practice. Total C3 and C4 immunoassays are available, while complement activity (CH50 lytic assay) is measured if a deficiency is suspected.

For all these reasons, a more detailed proteomic characterization of the complement cascade and its related regulation is warranted. **NRicher™ C** can provide an approximate 2-4X enrichment for all the circulating complement and complement-regulating proteins, see Table on next page. Using this, precision medicine biomarkers and therapeutic targets for modulating the innate immune response, in both acute and chronic disease, will be derived.

Protein Name	Uniprot Identity	Apprx Conc. µg/ml	Action
C1R (Complement C1r subcomponent, Classical)	P00736	100	Serine protease that combines with C1q and C1s to form C1, the first component of the classical pathway of the complement system.
C1S (Complement C1s subcomponent, Classical)	P09871	80	Serine protease that combines with C1q and C1r to form C1, the first component of the classical pathway of the complement system. C1r activates C1s, to activate C2 and C4.
Complement C1q subcomponent subunit A	P02745	60	C1q associates with the proenzymes C1r and C1s to yield C1, the first component of the serum complement system.
Complement C1q subcomponent subunit B	P02746	55	C1q associates with the proenzymes C1r and C1s to yield C1, the first component of the serum complement system.
Complement C1q subcomponent subunit C	P02747	50	C1q associates with the proenzymes C1r and C1s to yield C1, the first component of the serum complement system.
C2 (Complement C2)	P06681	20	Part of the classical pathway, cleaved by activated factor C1 into two fragments: C2b and C2a. C2a combines with C4b to form C3 convertase (classical, lectin)
Factor D (Complement factor D)	P00746	3	Cleaves Factor B when the latter is complexed with factor C3b, assists to activate C3 convertase of the alternate pathway
Factor B (Complement factor B)	P00751	320	Cleavage Product Bb combines with C3b to form C3 Convertase (Alternative). Fragment Bb forms complex with Properdin.
Complement C3	P01024	1,500, 000	Central role in the activation of the complement system, multi-functional sub-unit C3b triangulates complex with Properdin and Complement Factor B. Many different proteolytic sub-forms exist in the circulation.
Complement Factor H	P08603	500	Accelerates the decay of the complement alternative pathway C3 convertase (C3bBb), cofactor of the serine protease factor I
Complement Factor H related protein 1 (CFHR1)	Q03591	40	The dimerized forms have avidity for tissue-bound complement fragments and efficiently competes with the physiological complement inhibitor CFH.
Complement Factor H related protein 2 (CFHR2)	P36980	60	The dimerized forms have avidity for tissue-bound complement fragments and efficiently competes with the physiological complement inhibitor CFH
Complement Factor H related protein 4 (CFHR4)	Q92496		Might be involved in complement regulation
Properdin (CFP)	P27918	25	Properdin is present in plasma, and released from neutrophil granules upon stimulation. It is a positive regulator of the alternate pathway of complement, stabilizing the C3- and C5-convertase enzyme complexes.
Complement C4-A	P0C0L4	100	Non-enzymatic component of the C3 and C5 convertases, propagates the classical complement pathway, derived from proteolytic degradation of complement C4. The C4 protein derives from a simple two-locus allelic model, the C4A-C4B genes, that allows for an abundant variation in the levels of their respective proteins within a population.
Complement C4-B	P0C0L5	365	Non-enzymatic component of the C3 and C5 convertases, propagates the classical complement pathway, derived from proteolytic degradation of complement C4. The C4 protein derives from a simple two-locus allelic model, the C4A-C4B genes, that allows for an abundant variation in the levels of their respective proteins within a population.
Complement C4b-binding protein alpha chain	P04003	300	Cofactor for Factor I, accelerates decay of classical pathway C3 convertase. Also, a cofactor for Protein S in the coagulation pathway. In plasma, 60–70% of Protein S is bound to C4b-binding protein.
Complement C4b-binding protein beta chain	P20851	0.5	The beta chain of C4b-binding protein binds protein S.
Factor I (Complement factor I)	P05156	35	Serine protease controls complement by cleaving three peptide bonds in the alpha-chain of C3b and two bonds in the alpha-chain of C4b thereby inactivating these proteins. Essential cofactors for these reactions include factor H and C4BP in the fluid phase and membrane cofactor protein/CD46 and CR1 on cell surfaces.
MASP 1 (Mannan-binding lectin serine protease 1)	P48740		Activates MASP2 or C2 or C3
MASP 2 (Mannan-binding lectin serine protease 2)	O00187		Cleaves/Activates C2 and C4
MASP 3 (Mannan-binding lectin serine protease 3)	P48740		Cleaves/Activates Complement Factor D
MBL2 (Mannose-binding protein C)	P11226		Binds mannose, fucose and N-acetylglucosamine, activates the lectin complement pathway
Complement C5	P01031	60	C5 convertase initiates the assembly of the membrane attack complex (MAC).
Complement C6	P13671	60	Constituent of the membrane attack complex (MAC)
Complement C7	P10643	90	Constituent of the membrane attack complex (MAC)
Complement component C8 alpha chain	P07357	50	Constituent of the membrane attack complex (MAC)
Complement component C8 beta chain	P07358	50	Constituent of the membrane attack complex (MAC)
Complement component C8 gamma chain	P07360	20	Constituent of the membrane attack complex (MAC)
Complement C9	P02748	50	Constituent of the membrane attack complex (MAC)
Clusterin	P10909	60	A multi-functional protein, Clusterin along with Factor H, C4BP, CFHR1, and Vitronectin limit formation of complement activation products at cell surfaces.
Vitronectin	P04004	115	Inhibits terminal MAC.

Product	Size	Total serum/plasma samples processed	Item No.
NRicher™ C	10 Preps	10 x (25-50) µl samples	NRCO-10
NRicher™ C	50 Preps	50 x (25-50) µl samples	NRCO-50

NRicher™ Mx

General enrichment for all biofluids and tissue lysates

- Consumable chemically derived beads, species agnostic as they are not derived from antibodies
- Enrich low abundance proteomes from any source, from sera/plasma to cell lysates from both animals and humans, >90% Albumin removal
- Scaleable protocol from small to large sample volumes, from 10 to 500 µl, and low to high protein concentrations
- Enriched sub-proteome, for better signal quantitation between samples
- Does not require any specialized instruments, just a standard microfuge
- Bead format suitable for automation compatibility, please inquire
- On-Bead digestion for LC-MS analysis, or optional elution for any functional, enzymatic, or immunoassay analysis

NRicher™ Mx employs the use of a bead cocktail, which allows for one, rather than multiple LC-MS analyses to establish dynamic range compression, and better signal quantitation. **NRicher™ Mx** is thus an all-purpose proteomic enrichment product that can be used for any sample type, from biofluids to tissue lysates. It is compatible with up to 1% non-ionic detergent concentrations.

It is particularly useful for membrane proteins; targets of over 50% of all therapeutic drugs, membrane proteins perform a variety of functions including:

- Receptors which relay information between internal and external environments
- Transport of molecules and ions across the cell membrane
- Enzymatic/proteolytic function, and
- Cell adhesion

NRicher™ Mx beads provide excellent 2-3X enrichment of membrane proteins, most of which are not observable in neat serum.

Another example of **NRicher™ Mx** bead enrichment is α-Synuclein, a biomarker for Parkinsons Disease, observed by **NRicher™ Mx**, but not observed in the neat serum.

Gene	Uniprot	NRicher™ Mx	Neat serum
SNCA	P37840	1.1E+06 total signal	Not detected

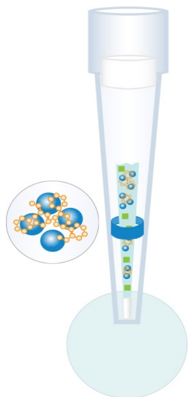
Product	Size	Total serum/plasma samples processed	Item No.
NRicher™ Mx	10 Preps	10 x (2-4) mg total protein samples	NIMX-10
NRicher™ Mx	50 Preps	50 x (2-4) mg total protein samples	NIMX-50

NRicher™ Beads Are Versatile to A Variety of Bead Processing Formats

Microfuge Spin-filter is our standard

Other formats compatible with the 50 um NRicher™ beads are:

High Throughput Automation Compatible INTip™ SPE (DPX Technologies) Format

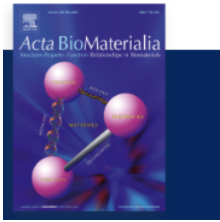


The INTip™ SPE tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. The tip-based formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. Please inquire for more information, as these formats are customized to the application and automation platform.

96-Well Vacuum or Pressure Filter Format

The NRicher™ beads can be readily processed in 96-well filter formats. Please inquire.

NRicher™ is an extended platform, derived from the same foundational surface chemistry as two previously successful products – **AlbuVoid™** and **HemoVoid™**. Some examples for the application of these products are:

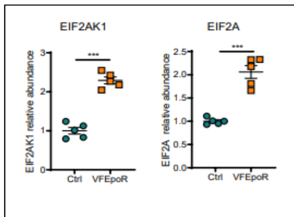


Jing, Lun, et al. "PROTEOMIC ANALYSIS IDENTIFIED LBP AND CD14 AS KEY PROTEINS IN BLOOD/BIPHASIC CALCIUM PHOSPHATE MICROPARTICLE INTERACTIONS." *Acta Biomaterialia* (2021).
Here, in a LC-MS/MS proteomic study, the article describes use of **HemoVoid™** and **AlbuVoid™** prior to LC-MS analysis, "...After **albumin depletion**, analysis of the significant deregulated proteins showed that **27 signaling pathways significantly changed in blood cells...**"

Vialaret, Jerome & Kadi, Sarah & Tiers, Laurent & O Flynn, Robin & Lehmann, Sylvain & Hirtz, Christophe. (2018). Albumin depletion of human serum to improve quantitative clinical proteomics. Current Topics in Peptide & Protein Research 19. 53-62. http://www.researchtrends.net/tia/abstract.asp?in=0&vn=19&tid=26&aid=6192&pub=2018&type=3. The article states "In comparison, methods using antibodies needed at least one-half day more. The albumin depletion method allowed to save precious time. ". The authors concluded that the **AlbuVoid™ depletion method proved to be faster and more cost-effective than antibody based methods**, and could be helpful for biomarker enrichment and detection in medical research.

Cell Reports

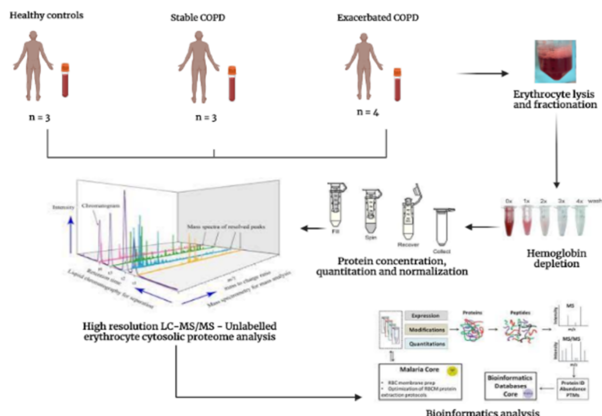
Jenull, Sabrina, et al. "The histone chaperone HIR maintains chromatin states to control nitrogen assimilation and fungal virulence." *Cell Reports* 36.3 (2021): 109406.
The article states for "Cell-free supernatants from 16 hours YNB-BSA (0.025% BSA) cultures grown at 30°C were used for Mass-Spec analysis. Collected supernatants were lyophilized and dissolved in 400 µl of water for **AlbuVoid™ treatment** for albumin depletion...**Albumin-free enriched secretory proteome** was eluted from beads".



Liu, Wenli, et al. "Erythroid lineage Jak2 V617F expression promotes atherosclerosis through erythrophagocytosis and macrophage ferroptosis." *The Journal of Clinical Investigation* (2022).
To explore underlying defects promoting oxidative changes in Jak2VF Red Blood Cells (RBC), unbiased proteomics profiling was conducted. The article states "...**haemoglobin removal and on-bead digestion**, ...was based on the protocol in **HemoVoid™** kit." Quantitative differentiation between sample biomarkers is demonstrated on the Table.

Klatt, Stephan, et al. "Optimizing red blood cell protein extraction for biomarker quantitation with mass spectrometry." *Analytical and Bioanalytical Chemistry* (2020): 1-14.
The article describes the advantage of HemoVoid™ in detection of low abundance proteins when comparing their amounts (in percent) between four alternative extraction conditions, stating "... Most peptides, following HemoVoid™ extraction, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols". With respect to potential biomarkers for Parkinson's Disease, the article states "For example, PRDX6 accounts for 0.4% of the total ion abundance after DOC (deoxycholate) extraction, **whereas following HV (HemoVoid™) extraction, this increases to 8%, a 20-fold enrichment**".

David L. Wang, Chuanguang Xiao, Guofeng Fu, Xing Wang and Liang Li. "Identification of potential serum biomarkers for breast cancer using a functional proteomics technology". Biomarker Research (2017) 5:11. The article states "The **most dramatic difference for enzyme activity detection in using the AlbuVoid™ for serum protein enrichment was demonstrated** ... Compared with the direct serum proteinase measurement, both the levels and species of proteases were increased significantly in the enriched serum sample. ..., **and it is necessary to use AlbuVoid™ to enrich these low level proteases to bring them to a high enough level to be detected**."



Das, Sonu, et al. "A journey to unravel the pathophysiology of stable and exacerbated Chronic obstructive pulmonary disease through erythrocyte proteomics: A combined mass spectrometry/bioinformatics approach." (2022).
Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disorder with high mortality. A label free relative quantification of erythrocyte cytosol proteome based on LC-MS/MS was performed on hemoglobin- depleted erythrocyte lysate samples of stable and exacerbated COPD, relative to healthy controls. The article states "**HemoVoid™, ... was used to remove hemoglobin from erythrocyte lysate samples to unmask low abundance...proteins.**" The article describes the observation of five highly enriched protein clusters in stable and seven in exacerbated COPD.

Conclusion

These examples demonstrate how **NRicher™** products can serve the two main goals of proteomics:

1. Discovery and coverage of the proteome to identify and monitor the signal of low abundance protein(s) or their modifications, that associate with disease, and
2. Targeted quantitative workflows, when the protein biomarkers of interest are known and enrichment increases efficiency and reproducibility of the measurements.

The simplified **NRicher™** workflows help to minimize variance or bias in the data, an important consideration when large cohorts are required to make conclusions. Minimizing technical variance is critical to measuring small but significant biological variance between samples. For targeted protein quantitation by MRM and related methods, enrichment of targets at the protein level, can improve target peptide signal to noise, as LC co-eluting non-target peptides can suppress target ion signals. Enrichment also can help minimize acquisition time, collectively improving overall throughput, cost, and productivity. For these reasons, combining sub-proteome enrichment with on-bead digestion is especially desirable. **NRicher™** is applicable to scalable formats, for high-throughput and automation. Eluates from **NRicher™** beads can also be used in orthogonal analyses, such as enzymatic/functional assays, 2DE, or ELISA/immunoassay to further validate discoveries. Finally, about 10% of plasma proteins do not appear to have high bead-binding activity, and these may be better monitored through our depletion products: AlbuSorb™, AlbuSorb™ PLUS, HemogloBind™, & NuGel™ HemogloBind™. Through a searchable knowledgebase of >2000 serum proteins, a selection of one or more **NRicher™** bead(s) and/or our depletion products can be investigated for specific protein targets of interest.

References
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Related Separations, Enrichment/Depletion & Sample Prep - All Product Categories
(https://www.biotechsupportgroup.com/Products-a-z_a/258.htm)

Albumin & IgG Removal
(https://www.biotechsupportgroup.com/Articles.asp?ID=451)

Lipid Removal and Clarification
(https://www.biotechsupportgroup.com/Articles.asp?ID=456)

Hemoglobin Removal
(https://www.biotechsupportgroup.com/Articles.asp?ID=452)

Sample Prep – Liquid Biopsy
(https://www.biotechsupportgroup.com/Articles.asp?ID=457)

Sample Prep – Glyco, Virus, Kinase, Aqueous Protein Crash/Metabolomics
(https://www.biotechsupportgroup.com/Articles.asp?ID=453)

Sample Prep – Mass Spectrometry
(https://www.biotechsupportgroup.com/Articles.asp?ID=432)

Sample Prep – Genomics
(https://www.biotechsupportgroup.com/Articles.asp?ID=455)

www.biotechsupportgroup.com