BSG

BIOTECH SUPPORT GROUP

NRicher™ Apo

Enrichment of Apolipoproteins

- Consumable chemically derived beads, species agnostic as they are not derived from antibodies
- Enrich Apolipoproteins from sera or plasma from both animals and humans, >90% Albumin removal
- 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 alternative digestion and/or other functional, enzymatic, or immunoassay analysis

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.

NRicher™ Apo Enrichment of Apolipoproteins

	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

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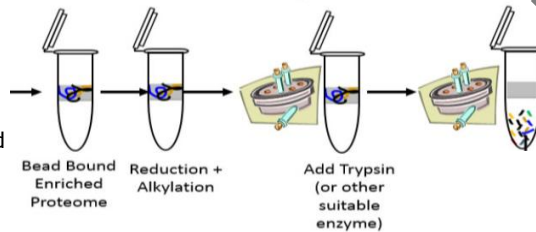
NRicher™ workflow

High abundance (i.e., Albumin) proteins selectively pass or void through

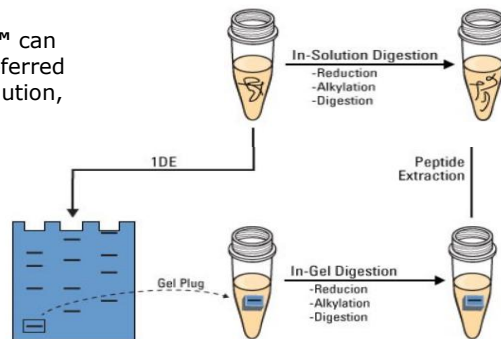the beads, concentrating and enriching sub-proteomes on the beads

Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with the **NRicher™** products

OR

Eluate from **NRicher™** can be digested by any preferred method, In-Gel, In-Solution, FASP, etc.

Digest Options

Bead Bound Enriched Proteome — Reduction + Alkylation — Add Trypsin (or other suitable enzyme)

In-Solution Digestion
-Reduction
-Alkylation
-Digestion

1DE

Gel Plug

In-Gel Digestion
-Reducion
-Alkylation
-Digestion

Peptide Extraction

LC-MS

Eluates from **NRicher™** beads can be applied to other common analyses:

- Enzymatic/Functional assays
- 2DE
- ELISA/immunoassay

The NRicher™ Workflow. All **NRicher**™ beads are 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 including:

>Bead-Assisted Sample Prep (BASP™), whereby reduction, alkylation and digestion are performed on the bead-bound proteome, without the use of detergents, seamlessly integrating to LC-MS analysis,

OR

>Optional Elution to off-bead digestion (i.e., FASP), or other common functional or immunoassay analyses

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

Processes 25-50 µl serum per prep. It is recommended that the volume be optimized for the application.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe, or microfuge-type filter before beginning the prep.

Depending upon the quality of the sample, centrifugation times can be adjusted to increase g's or time, sufficient to process the sample through the beads.

The protocol can be scaled up or down proportionally to adjust for different volumes. The bead amount can be adjusted to accommodate more or less apolipoprotein capture.

Items Required	10 Prep	50 Prep	Reagent
NRicher™ Apo Beads	0.25 gram	1.25 gram	Supplied
Binding Buffer NRBB (0.05M HEPES, pH 6.0)	5 ml	25 ml	Supplied
Wash Buffer NRWB (0.05M HEPES, pH 7.0)	12 ml	60 ml	Supplied
Elution Buffer NREB (0.25M Tris + 0.5M NaCl, pH 9-10)	3 ml	15 ml	Supplied
Spin-filter & tube assemblies*	10	50	Supplied
DTT, Iodoacetamide, Trypsin and Formic Acid, 50% Acetonitrile (ACN)			Not Supplied

*Additional Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

If there are any questions about compatibility or substitution with other buffers, please contact us.

Protocol For Enrichment of Apolipoproteins from Serum/Plasma & On bead Digestion For LC-MS Analysis

Optional Elution Protocol is included for Off-bead digestion or any functional, enzymatic, or immunoassay analysis

In bold are the **NRicher™** kit components.

1. **BEAD CONDITIONING.** Weigh out 25 mg of **NRicher™ Apo** beads in a spin-filter. Add 150 µl of **Binding Buffer NRBB.** Vortex for 5 minutes at room temperature followed by centrifugation for 2 minutes at 1,000 g's. Discard the filtrate. Repeat step-1.

2. **SAMPLE PROCESSING.** Add 200 µl of **Binding Buffer NRBB** to beads followed by (25 to 50) µl of the Serum to the beads. Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000 g's.

3. To the beads, add 250 µl of **Wash Buffer NRWB.** Vortex for 5 min and centrifuge for 4 minutes at 5,000 g's. Discard the **Wash** filtrate.

4. Repeat Wash Step-3.

5. **After discarding the wash from step 4, the NRicher™ beads contain the enriched sub-proteome. As an option for LC-MS sample preparation, the bead assisted on-bead digestion protocol (BASP™) is provided starting on step 6, see box below.**

   OPTIONAL BEAD ELUTION. To the beads, add 300 µl of **Elution Buffer NREB.** Vortex or mix thoroughly for 10 min and centrifuge for 4 minutes at 5,000 g's. Recover the filtrate as the eluted sub-proteome (0.25M Tris + 0.5M NaCl, pH 9.0-10.0), suitable for further analysis.

The bead assisted on-bead digestion protocol (BASP™) is provided below. The digest buffer is **Wash Buffer NRWB** (0.05M HEPES, pH 7.0). Comparable buffers (0.02-0.10M, pH 6-7) can be used. Higher pH buffers are not recommended.

6. Using **Wash Buffer NRWB**, prepare to 10mM of DTT concentration, and add 100 µl to the **NRicher™** beads and vortex for 10 minutes and incubate for 30 minutes at 60C.

7. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes.

8. Centrifuge 4 minutes at 5,000 g's, and discard filtrate. Rinse the bottoms of the spin-filter tubes with 500 µl of 50% ACN, **Wash Buffer NRWB** twice, to remove any traces of the filtrate.

9. Add 8 µg trypsin in 100 µl **Wash Buffer NRWB** to the **NRicher™** beads and keep at 37˚C for a minimum 4 hours to maximum overnight. Overnight is recommended to start with. In select targeted circumstances, 2 hours may be sufficient.

10. Centrifuge 4 minutes at 5,000 g's, and retain digested peptides filtrate.

11. To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge 4 minutes at 5,000 g's, and combine this volume with volume from step 10.

12. Total is about 250µl. Prepare to desired final concentration. Store at -80°C until LC-MS/MS.

For Targeted Proteomics

NRicher™ Bead Platform Provides Unique Sub-Proteome Biases And Fit For Purpose Opportunities for Targeted LC-MS Quantification

Learn more at: https://www.biotechsupportgroup.com/category-s/335.htm

References

Swapna LS, Stevens GC, Sardinha-Silva A, Hu LZ, Brand V, Fusca DD, et al. (2024) ToxoNet: A high confidence map of protein-protein interactions in Toxoplasma gondii. PLoS Comput Biol 20(6): e1012208. https://doi.org/10.1371/ journal.pcbi.1012208
The article states we used affinity beads (NuGel PROspector) to pre-enrich Toxoplasma gondii lysate to capture five distinct subproteomes. [Note: NuGel PROspector beads are now part of the **NRicher™** platform.]. When comparing the 5 different subproteomes, there is clearly different selection biases amongst the 5 surface chemistries. Also, many of the proteins observed from the **NRicher™** beads, were not observed in the Ion exchange fractions demonstrating the importance of combining different modes (ionic, hydrophobic, etc.) of separation to alter selection properties, and consequently improving overall proteome coverage.

Efficiencies gained in targeted serum proteomics using NRicher Beads – simplified and diversified workflows for sub-proteome and biomarker enrichment – Poster HUPO World Congress 2024
After **NRicher™** sample prep, target peptides have highly enhanced spectral signal. **NRicher™** sub-proteome enrichment can minimize acquisition time, collectively improving overall throughput, cost, and productivity. Specific target peptides that report functional PTMs and amino acid variant regions promise insights and potential multiplex biomarkers for disease.
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/HUPO%202024%20Efficiencies%20Gained.pdf

Wan, C., Borgeson, B., Phanse, S. et al. Panorama of ancient metazoan macromolecular complexes. Nature 525, 339–344 (2015). hXps://doi.org/10.1038/nature14877
Six different **NRicher™** beads (described with an old tradename PROspector) were used as an enrichment step in the overall workflow; about twice the number of observations and annotations became possible. This further validates that the sub-proteome bias characteristics of the **NRicher™** surface chemistry platform can simplify complex proteomes into enriched sub-proteomes with efficiencies suitable for deep functional proteome characterization.

Whitepaper - NRicher™: A Low Abundance Proteome Enrichment Platform With Seamless Integration of On-Bead Digestion
The NRicher™ Advantage is described: • 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
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/BiotechSupportGroup-NRicher-Whitepaper.pdf

NRicher : Family Specific Enrichment For Targeted Proteomics – Poster US HUPO 2024
The need for new biomarkers to support personalized healthcare, has fostered numerous proteomic innovations. Still, a number of challenges remain. One is the preponderance of high abundance proteins and, concurrently in targeted proteomic

workflows, efficiency and consistency in quantifying target peptides from different sample cohorts. This is in part due to the changing landscape of proteins/peptides not associated with the selected targets. A solution for both these challenges is now available through a suite of products called NRicher .
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/NRicher%20poster%20small.pdf

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

In addition to standard spin-filter formats, other formats compatible with the 50 μm NRicher™ beads are:

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

Aspirate and dispense cycles mix NRicher™ beads and solutions

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.

CONTACT US
We welcome your questions and comments regarding our products.
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