



## 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 any 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

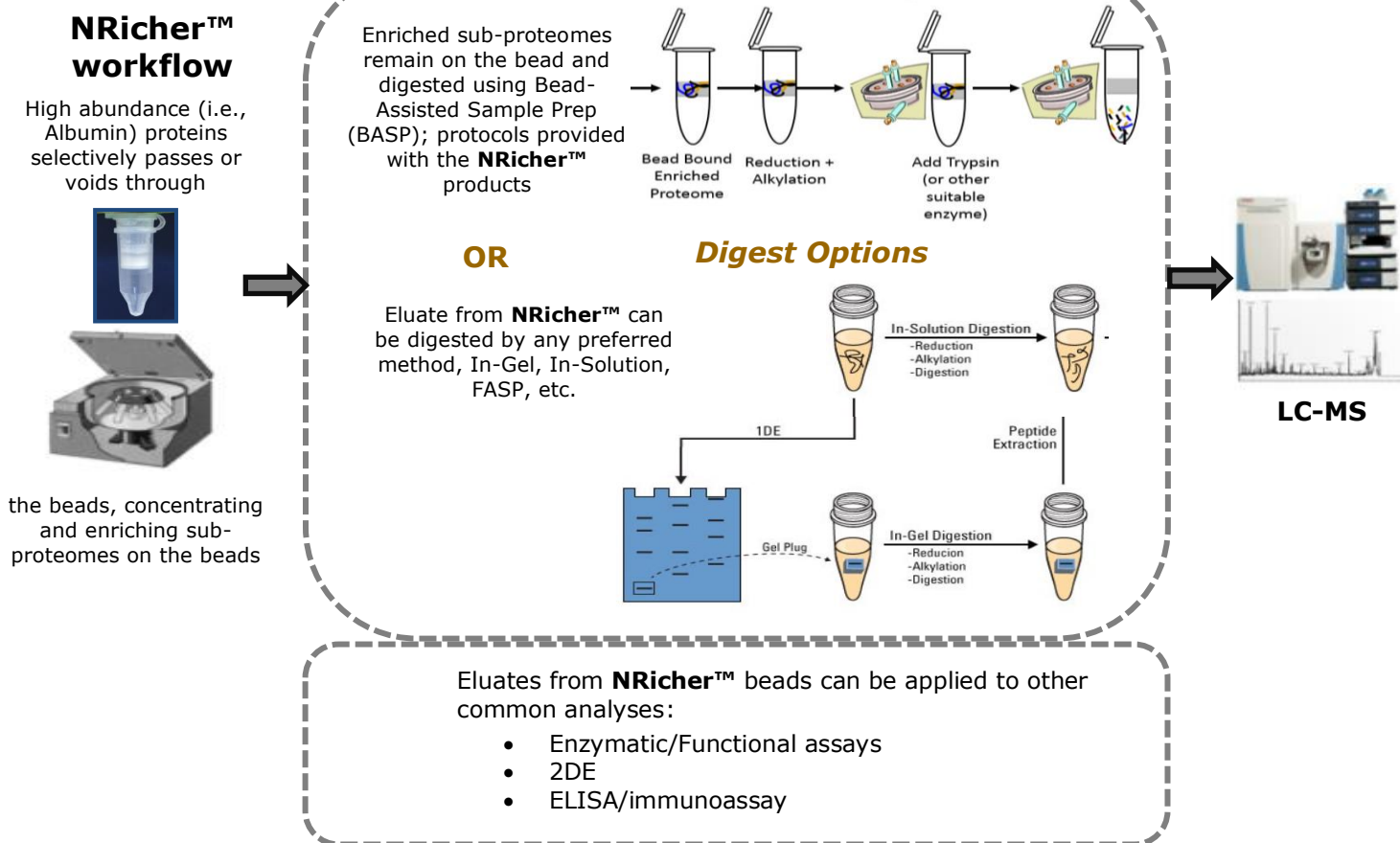
Bead Enrichment Factor Relative to Neat = (% of Gene Specific Signal relative to Total Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Total Signal from Neat)

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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**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



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Product	Size	Total serum/plasma samples processed	Item No.
<b>NRicher™ Apo</b>	10 Preps	10 x (25-50) $\mu$ l samples	NAPO-10
<b>NRicher™ Apo</b>	50 Preps	50 x (25-50) $\mu$ l samples	NAPO-50

**Processes 25-50  $\mu$ l 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  $\mu$ m syringe, or microfuge-type filter before beginning the prep.

The centrifugation time may vary, adjust as necessary to get complete filtration 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.

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

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  $\mu$ m 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**



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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 5,000-10,000 rpm (2,000-8,000xg). 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 5,000-10,000 rpm (2,000-8,000xg).
3. To the beads, add 250 µl of **Wash Buffer NRWB**. Vortex for 5 min and centrifuge for 4 minutes at 5,000-10,000 rpm (2,000-8,000xg). 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 5,000-10,000 rpm (2,000-8,000xg). 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 at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, 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 at 5,000-10,000 rpm (2,000-8,000xg) for 5 minutes, and retain digested peptides filtrate.
11. To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, centrifuge at 5,000-10,000 rpm (2,000-8,000xg) for 5 mins., 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.



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

#### Microfuge Spin-filter is our standard



#### Other formats compatible with the 50 µm NRicher™ beads are:

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

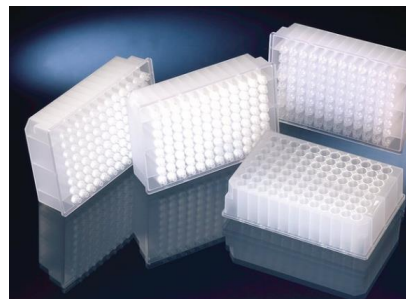
Aspirate and dispense  
cycles mix NRicher™ beads  
and solutions



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.





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### **Related Separations, Enrichment/Depletion & Sample Prep - All Product Categories**

([https://www.biotechsupportgroup.com/Products-a-z\\_a/258.htm](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>)

## **CONTACT US**

**We welcome your questions and comments regarding our products.**

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