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BIOTECH SUPPORT GROUP

NRicher™ Ig

Enrichment of all isotypes and subclasses of Immunoglobulins

- Consumable chemically derived beads, species agnostic as they are not derived from antibodies
- Enrich circulating immune complexes 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

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.

NRicher™ Ig Enrichment of Immunoglobulins

	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)

Human serum immunoglobulins comprise several classes IgG, IgA, IgM, IgD & IgE. 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. 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.

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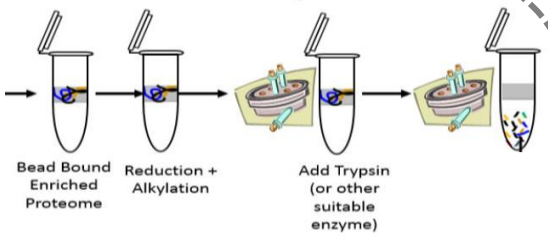
NRicher™ workflow

High abundance (i.e., Albumin) proteins selectively passes or voids through

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

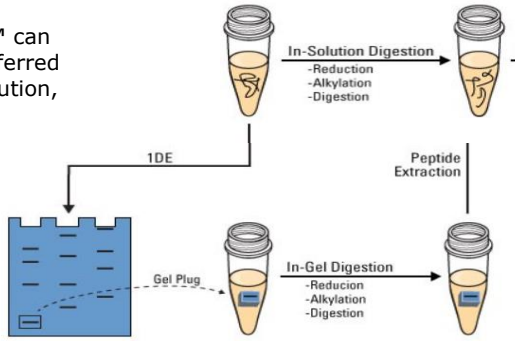
Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with the **NRicher™** products



OR

Digest Options

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



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

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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

Processes 25-50 µl serum per prep. It is recommended that the volume be optimized for the application. For example, when recovery is paramount for quantitative targeted SRM/MRM enrichments investigations, smaller volumes may be better. However, for increased total protein/antigen annotations, larger volumes may be optimal.

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.

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 Immunoglobulin capture.

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

Items Required	10 Prep	50 Prep	Reagent
NRicher™ Ig 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 Immunoglobulins 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™ IG** beads in a spin-filter. Add 150 µl of **Binding Buffer VRBB.** 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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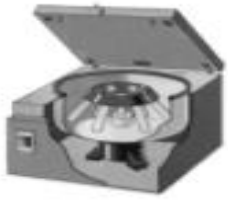
NRicher™ Beads Are Versatile to A Variety of Bead Processing Formats

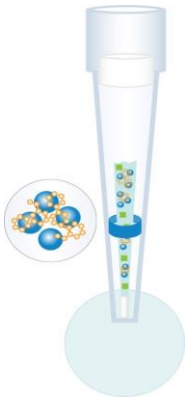
Microfuge Spin-filter is our standard



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)

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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