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BIOTECH SUPPORT GROUP

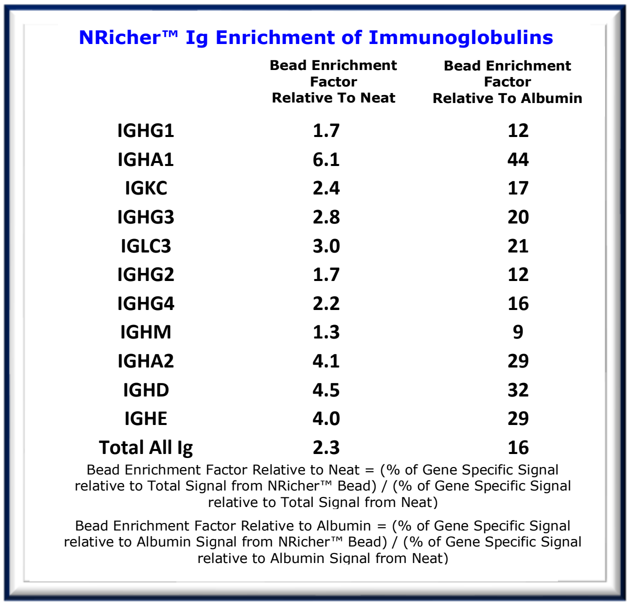
# NRicher™ Ig

## *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 alternative digestion and/or other 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)

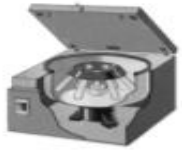
Bead Enrichment Factor Relative to Albumin = (% of Gene Specific Signal relative to Albumin Signal from NRicher™ Bead) / (% of Gene Specific Signal relative to Albumin Signal from Neat)

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.

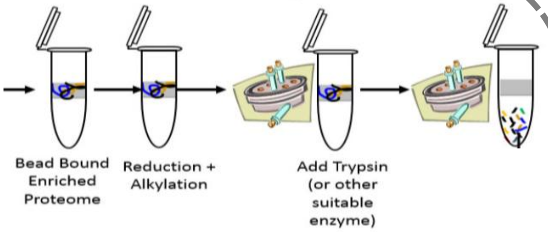
Nevertheless, the ability to enrich circulating immune complexes from sera or plasma from both animals and humans with high yield and without selective loss of isotypes or subclasses can provide more comprehensive profiles. **NRicher™ Ig** can provide such enrichment for all immunome profiling methods. 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.

## NRicher™ workflow

High abundance (i.e., Albumin) proteins selectively pass or void through the beads, concentrating and enriching sub-proteomes on the beads



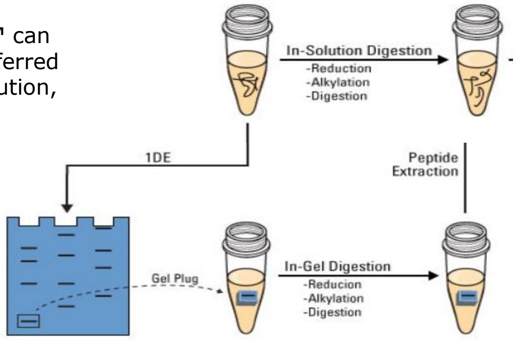
Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with the **NRicher™** products



**OR**

***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 |
| **NRicher™ Ig** | Please inquire about bulk quantities | | |

**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.

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 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<br>Refrigerate upon arrival |
| Wash Buffer NRWB (0.05M HEPES, pH 7.0) | 12 ml | 60 ml | Supplied<br>Refrigerate upon arrival |
| Elution Buffer NREB (0.25M Tris + 0.5M NaCl, pH 9-10) | 3 ml | 15 ml | Supplied<br>Refrigerate upon arrival |
| 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

1. **Bead Conditioning.** Weigh out 25 mg of **NRicher™ IG** 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 at 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.

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## 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

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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