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NRicher™ Mx

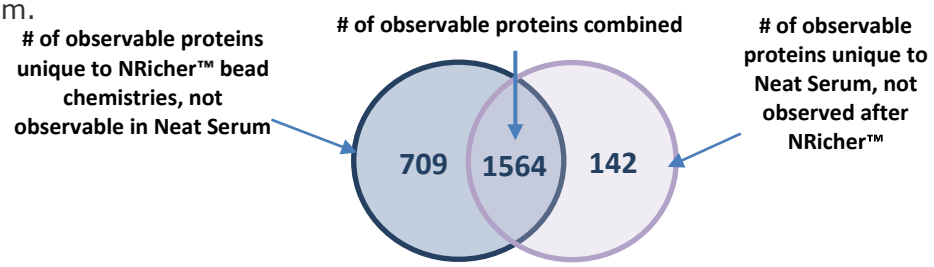
General Enrichment for All Biofluids and Tissue Lysates, Soluble Membrane Proteins

- 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
- Especially biased towards soluble membrane proteins
- 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

NRicher™ Mx employs the use of a bead cocktail, which allows for one, rather than multiple LC-MS analyses to establish dynamic range compression. **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 (i.e., RIPA Buffer).
It is particularly useful for soluble membrane proteins, derived from ectodomain shedding. 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, and
- Adhesion

NRicher™ Mx beads provide excellent 2-5X enrichment of soluble membrane proteins, most of which are not observable in neat serum.



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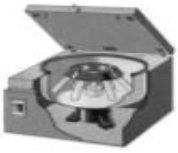
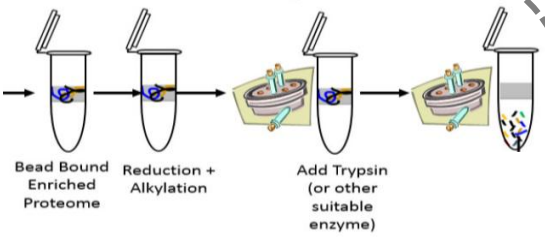
NRicher™ workflow

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



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

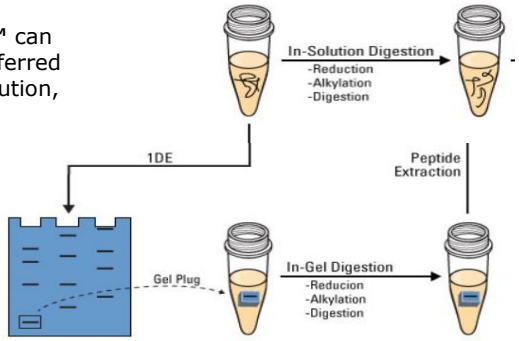
Enriched sub-proteomes remain on the bead and digested using Bead-Assisted Sample Prep (BASP); protocols provided with the **NRicher™** products



OR

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™ 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

Processes 25-100 µl serum per prep, or 2-4 mg total protein from cell lysates (diluted to ≤0.1% non-ionic detergent). If the cell lysates are dilute, follow the cell lysate protocol, step # 2 in protocol to follow. 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 loads/volumes may be better. For discovery and coverage, larger load/volumes may be better.

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 proteome capture.

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

Items Required	10 Prep	50 Prep	Reagent
NRicher™ Mx Beads	0.25 gram	1.25 gram	Supplied
Binding Buffer NRBB (0.05M HEPES, pH 6.0)	60 ml	300 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 Low Abundance Proteins, & 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. **SAMPLE PROCESSING.**

FOR SERUM OR BIOFLUIDS.

1a. Weigh out 25 mg of **NRicher™ Mx** 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.

1b. Add 200 µl of **Binding Buffer NRBB** to **NRicher™** beads followed by (25 to 100) µl Serum, Plasma or other biofluid, to the beads. Vortex or mix thoroughly for 10 min and then centrifuge for 4 minutes at 5,000 g's. Discard the filtrate.

FOR CELL LYSATES UP TO 5 ML (≤0.1% NON-IONIC DETERGENT), CONTAINING 2-4 MG TOTAL PROTEIN.

1a. To clarified cell lysate, add 5 ml **Binding Buffer NRBB** in 15 ml centrifuge tube.

1b. Add 25 mg **NRicher™** beads and vortex for 25 minutes. Note: Vortex sufficiently so that the beads do not settle at the bottom of the centrifuge tube.

1c. Allow the beads to settle for 10 minutes. Decant or pipette off the supernatant.

1d. Using a wide bore pipette, transfer **NRicher™** beads to the supplied spin-filter tube assembly. Note: if all beads do not transfer, use additional **Binding Buffer NRBB** (approximately 1 ml) to resuspend & transfer again.

1e. Centrifuge for 4 minutes at 5,000 g's. Discard the filtrate.

2. To the **NRicher™** 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.
3. Repeat Wash Step-2.
4. **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.

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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.

5. 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.
6. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes.
7. 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.
8. 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.
9. Centrifuge 4 minutes at 5,000 g's, and retain digested peptides filtrate.
10. To further extract remaining peptides, add 150 µL 10% formic acid, vortex 10 min, 4 minutes at 5,000 g's, and combine this volume with volume from step 10.
11. 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

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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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