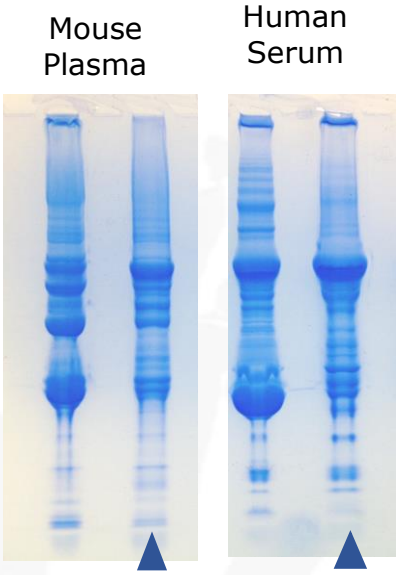
BIOTECH SUPPORT GROUP

NuGel™ NRicher™ Mx

Low Abundance Proteome Enrichment

- Enriches & normalizes sub-proteomes
- Compress proteome concentrations
- Species and tissue agnostic
- Composite of the **NRicher™ 6** mixed mode beads
  - In a rigorous examination of protein complexes, about twice the number of observations were made possible through sub-proteome bias characteristics of **NRicher™ 6** {doi:10.1038/nature14877}
- Suitable for small volume mg scale preps



NRicher™ Mx
Eluates

BSG has developed a new method for proteome separations based on chemically derived weak affinity or imperfect fit interactions. Without the use of antibodies, progressive displacement allows the beads to bias for or against certain proteins, without compromising protein integrity.

The related **NRicher™** (**6** & **Mx**) products are adaptable to any measurable protein function from any sample type, to aid in the discovery of new biomarkers. They support all functional, activity-probe, chemical and top-down proteomic applications.

- Separations readily compatible with virtually all proteomic interrogations
- Microtube kit, simple bind/wash/elute protocols
- No specialized instruments, or HPLC required
- Disposable, no column regeneration
- Tryptic digestion or enzyme assay can be 'on-bead'
- Universal, species and tissue type agnostic

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Product	# of preps*	Item No.
NuGel™ NRicher™ Mx	10	SRPRO-10
NuGel™ NRicher™ Mx	50	SRPRO-50

*Each prep processes approximately 0.5-1.0 mg total protein

The **NuGel™ NRicher™ Mx** product kit includes all surface reagents, binding and elution buffers and associated separations protocols. Each prep processes approximately 0.5-1.0 mg total protein, and produces eluate sub-proteomes in 60 µl volumes in less than 1 hour. Efficient low abundance proteome enrichment and protein compression may require higher loads, and will vary with sample type and user requirements.

Kit Contains:	NuGel™ NRicher™ Mx 10	NuGel™ NRicher™ Mx 50
NuGel™ NRicher™ Mx beads	150 mg	750 mg
***PRO*-BB** Binding Buffer, pH 6	3 ml	15 ml
PRO-WB Wash Buffer pH 7	5 ml	25 ml
***PRO*-EB** Elution Buffer, pH 1	1 ml	5 ml
Spin-X microfuge filters	10	50

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Low Abundance Proteome Enrichment

Protocol

Step 1 - Sample Preparation: The protocol is based on 100 µl of tissue homogenates with a soluble protein content in the 5 – 15 mg/ml range, per prep. Larger volumes of lower protein content can also be used for load but the total protein content applied should be in the range of 0.5 – 1.5 mg. For serum/plasma, we recommend at least 25 µl be applied.

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

It has not been evaluated on membrane or insoluble protein content, but it is compatible with up to 0.1% Triton X-100. For best results, pH for samples should be in the range of 6-7.

Step 2 - Surface Preparation. The **NuGel™ NRicher™ Mx** reagent is supplied in dry powder form. **Weigh out 15 mg for each prep and place into the Spin-X filters provided.** Before using, tap each to ensure powders are at the bottom of the filter cup.
1) Add 100 µl of **PRO-BB binding buffer** to each **reagent powder** and mix for 3 minutes.
2) Centrifuge at [5,000-7,000]g's for 4 min. and discard the flow-through.

For Steps 3 & 4. All centrifugations are for 4 minutes at 9,000 g's.

Step 3 - Separations.
1) Add 100 µl of **PRO-BB binding buffer** and 100 µl (or adjusted volume as stated above) sample, to each prep (from Step 2). Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard filtrate.
2) Add 250 µl of **PRO-WB wash buffer** to each surface as a wash. Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard filtrate.

Step 4 – Low abundance sub-proteome elution.
To elute the bound sub-proteome, use 60 µl **PRO-EB Elution buffer**. Mix to homogeneously resuspend. Shake the sample for 10 minutes. Centrifuge. Collect eluate filtrates for analyses.

Note: On-bead digestion can be applied to all **NuGel™**-based including **NRicher™ Mx**. Please contact our office for details.

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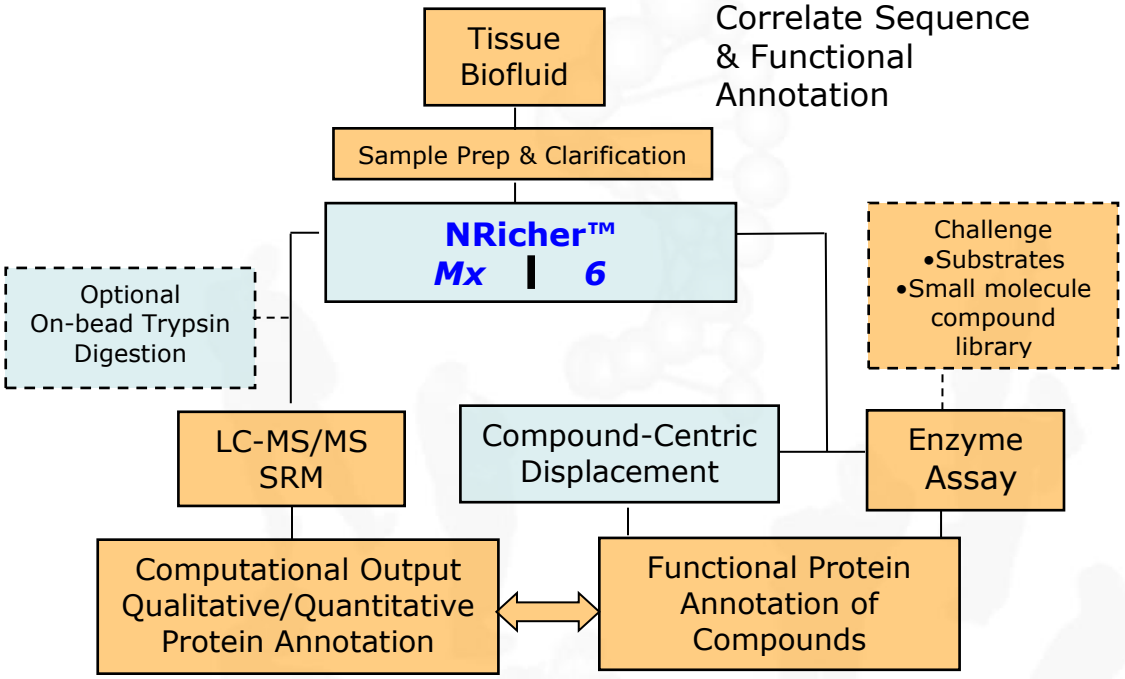
Related Functional Proteomic Product - NuGel™ NRicher™ 6

The related **NRicher™** (**6** & **Mx**) products are adaptable to any measurable protein function from any sample type, to aid in the discovery of new biomarkers. They support all functional, activity-probe, chemical and top-down proteomic applications.

NuGel™-based Functional Proteomics Products in Proteomic Workflows



Functional proteomics can help optimize drug candidates to tissue-specific expression of isoforms, gauge promiscuity, elucidate mechanism of action and identify biomarkers

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References

C Wan, B Borgeson, S Phanse, F Tu, K Drew, G Clark, et al. Panorama of ancient metazoan macromolecular complexes. Nature Volume:525, Pages:339–344 Date published:(17 September 2015). doi:10.1038/nature14877

Two of BSG products, **NRicher™ 6** and **HemogloBind™**, were able to contribute to this rigorous examination of protein complexes. When our products were used as a pretreatment 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 **NRicher™ 6** can simplify complex proteomes into less complex sub-proteomes with efficiencies suitable for deep functional proteome characterization. Furthermore, this study demonstrated the importance of a key feature implicit to all of our products; that is the maintenance of functional and structural integrity after separations. Without that particular feature, these additional observations would not have been possible.

Matthew P. Kuruc, Swapan Roy. The Functional & Chemical Proteomics Handbook

Oka, Amita R., Matthew P. Kuruc, Ketan M. Gujarathi, and Swapan Roy. "Functional Proteomic Profiling of Phosphodiesterases Using SeraFILE Separations Platform." International Journal of Proteomics 2012 (2012).

The surface chemisties trademarked here as SeraFILE in this article are now trademarked as the product **NRicher™ 6**.

Functional proteomic profiling can help identify targets for disease diagnosis and therapy. Available methods are limited by the inability to profile many functional properties measured by enzymes kinetics. The functional proteomic profiling approach proposed here seeks to overcome such limitations. It begins with surface-based proteome separations of tissue/cell extracts, using **NRicher™ 6**, a proprietary protein separations platform. Enzyme kinetic properties of resulting subproteomes are then characterized, and the data integrated into proteomic profiles. As a model, **NRicher™ 6**-derived subproteomes of cyclic nucleotide-hydrolyzing phosphodiesterases (PDEs) from bovine brain homogenate (BBH) and rat brain homogenate (RBH) were characterized for cAMP hydrolysis activity in the presence (challenge condition) and absence of cGMP. Functional profiles of RBH and BBH were compiled from the enzyme activity response to the challenge condition in each of the respective subproteomes. These results demonstrate that the proposed methods provide a means to simplify intersample differences, and to localize proteins attributable to sample-specific kinetic responses. It can be potentially applied for disease and non-disease sample comparison in biomarker discovery and drug discovery profiling.

CONTACT US

We welcome your questions and comments regarding our products.

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