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NuGel™ NRicher™ 6 Kit

Functional proteomics separations and enrichment kit

- All Top-down proteomic applications
- Efficiently produce up to 12 differentiated subproteomes with uncompromised functional and structural integrity
- Generate characteristic functional molecular profiles for comparison and discovery
- Enrich functional biomarkers for sequence and structural annotation without antibodies or bioengineering
    - 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}
- Investigate and compartmentalize drug response from natural sources
- Kit includes 6 mixed mode surface chemistries per prep

Functional proteomic annotation complements conventional sequence annotation while supporting the study of mechanism of action and drug promiscuity. Furthermore, the subtleties of protein attributes, when the same or similar underlying sequence can have multiple conformations and functions, and when different sequences sometime perform the same or similar functions, are now open to investigation.

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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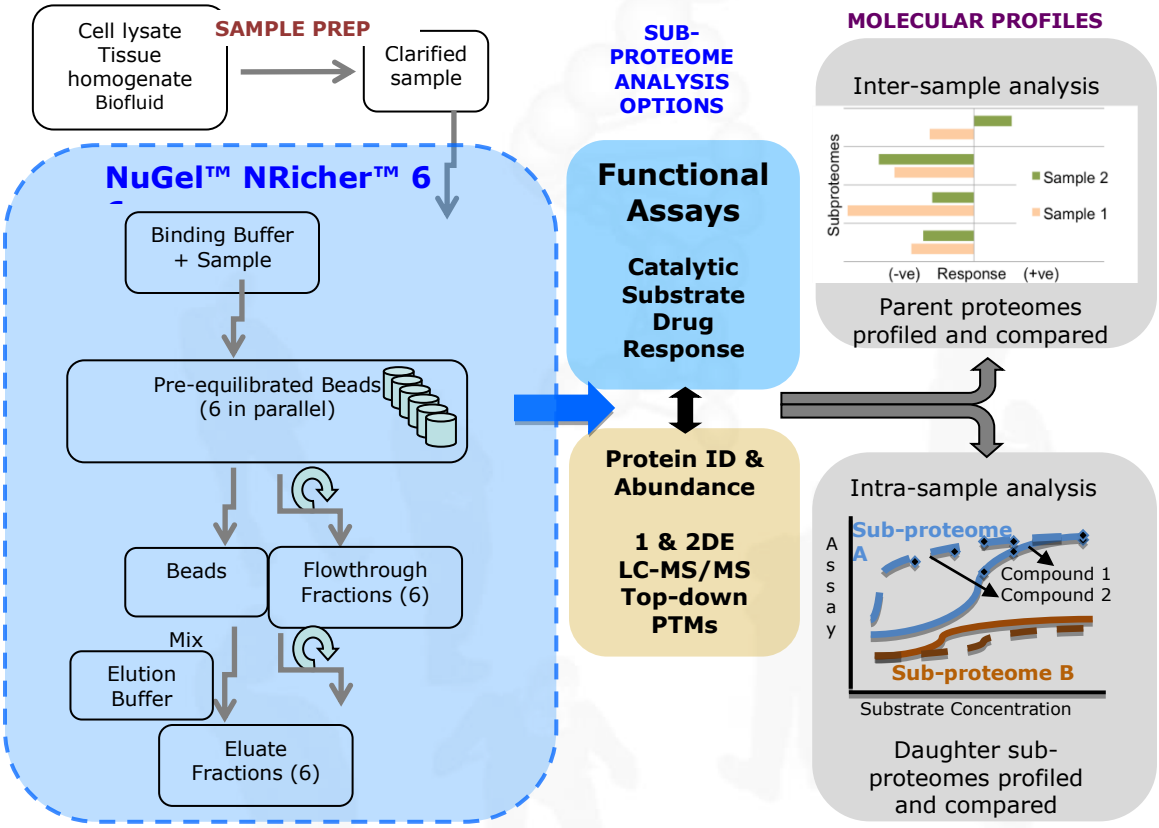
Product	# of preps*	Item No.
NuGel™ NRicher™ *6*	5 Preps	SR610-5
NuGel™ NRicher™ *6*	25 Preps	SR610-25

*Based on processing 0.5-1.0 mg total protein



The **NuGel™ NRicher™ 6** product kit includes 6 mixed mode bead surfaces, binding and elution buffers and associated separations protocols. Each prep processes approximately 0.5-1.0 mg total protein, and produces 12 daughter sub-proteomes in 60 µl volumes in less than 1 hour.

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Kit Contains:	NuGel™ NRicher™ *6* 5 Preps	NuGel™ NRicher™ *6* 25 Preps
***PRO*- (A,B,C,L,N,R)** reagent powders	75 mg each reagent	375 mg each reagent
***PRO*-BB** Binding Buffer, pH 6	15 ml	75 ml
PRO-WB Wash Buffer pH 7	10 ml	50 ml
***PRO*-EB** Elution Buffer, pH 10	2 ml	10 ml
Spin-X microfuge filters	30	150

Refrigerate upon arrival.

Protocol

Step 1 - Sample Preparation: The protocol is based on 150 µl of tissue homogenates with a soluble protein content in the 5 – 15 mg/ml range, per prep. It has not been evaluated on membrane or insoluble protein content, but it is compatible with up to 0.1% Triton X-100. Larger volumes of lower protein content can also be used. To accommodate different protein loads, sample volume can be adjusted. For best results, pH for samples should be in the range of 6-7.

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.

Step 2 - Surface Preparation.
Surface reagents are supplied as dry powders. **Reagents are labeled *PRO*-A, *PRO*-B, *PRO*-C, *PRO*-L, *PRO*-N, *PRO*-R. Weigh out 15 mg of each reagent, 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 100µl of ***PRO*-BB binding buffer** to each **reagent powder** and mix for 3 minutes.
2) Centrifuge at [5,000-7,000]xg for 4 min. and discard the flow-through.

Step 3 - Separations. All centrifugations are at 10,000xg for 4 min.
1) Add 25 µl of ***PRO*-BB binding buffer** and 25 µl of sample to each **reagent powder** (from Step 2). Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and collect the filtrate as "flow-through" fractions.
2) Add 200 µl of ***PRO*-WB binding buffer** to each surface as a wash. Mix until homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard filtrate. For LC-MS applications, on-bead digestion protocols are available, contact technical support for details.
3) Add 60 µl of ***PRO*-EB** elution buffer to each surface. Mix to homogeneously resuspend. Shake the sample for 10 minutes. Centrifuge. Collect filtrates as eluate fractions for analyses.

For optimal results, the volumes may need to be adjusted up or down to account for differences in specific activity and other sample matrix factors. The elution buffer is pH 10, so

activity measurements must compensate for either higher pH, dilutions to neutrality, or buffer exchange. For profile characterization of activity, we recommend that all fractions be protein normalized. In cases where eluate fractions have much lower protein content than the flow-through fractions, normalize the protein content of flow-through fractions and eluate fractions independently.

On-Bead Digestion Protocols
With greater interest in the proteomics community for better workflows and performance for LC-MS analyses, "on-bead" proteolytic digestion protocols can be applied to **NuGel™ NRicher™ 6**. Please contact technical support for more information.

Related Functional Proteomic Product - NRicher™ Mx Kit

Functional proteomics relies in part, on the functional or structural features of intact, non-denatured proteins. While the terminology can often overlap, chemical and affinity-based proteomic profiles can be considered a subset of functional proteomics. Both **NRicher™ Mx & NuGel™ NRicher™ 6** support functional and chemical proteomics and can:

- Optimize drug compounds
- Survey compound promiscuity
- Deconvolute targets, elucidate mechanism of action
- Identify phenotypic biomarkers

The **NuGel™ NRicher™ 6** kit includes the same 6 mixed mode chemistries within the composite **NRicher™ Mx** reagent. It can be used to deconstruct separations from **NRicher™ Mx** for optimal biomarker enrichment. Contact technical support for details.

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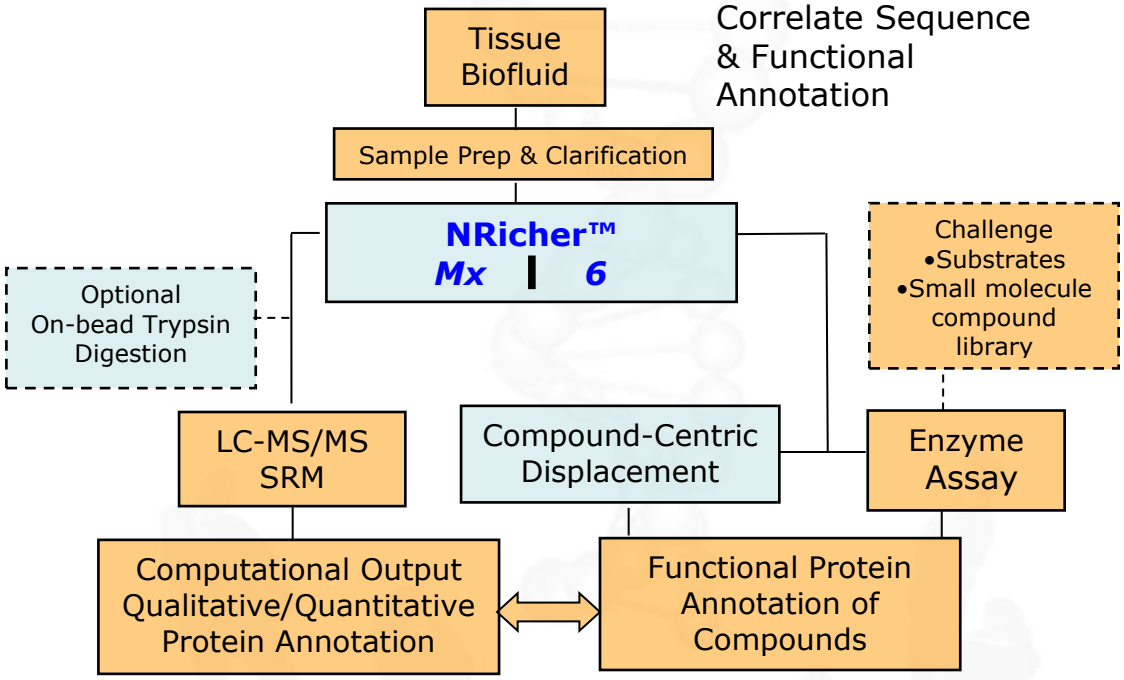
NuGel™-based Functional Proteomics Products in Proteomic Workflows



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 chemistries 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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