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NuGel™ NRicher™ 6 Kit

Functional proteomics separations and enrichment kit

- 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
- Investigate and compartmentalize drug response from natural sources
- Kit includes 6 mixed mode surface chemistries per prep

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.

Thus, functional proteomic annotation may complement 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.

A new functional proteomics separations toolset based on the NuGel™ passivated porous silica platform, can be used in unrestricted workflow strategies, so as to sift through these biological complexities.

- 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

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

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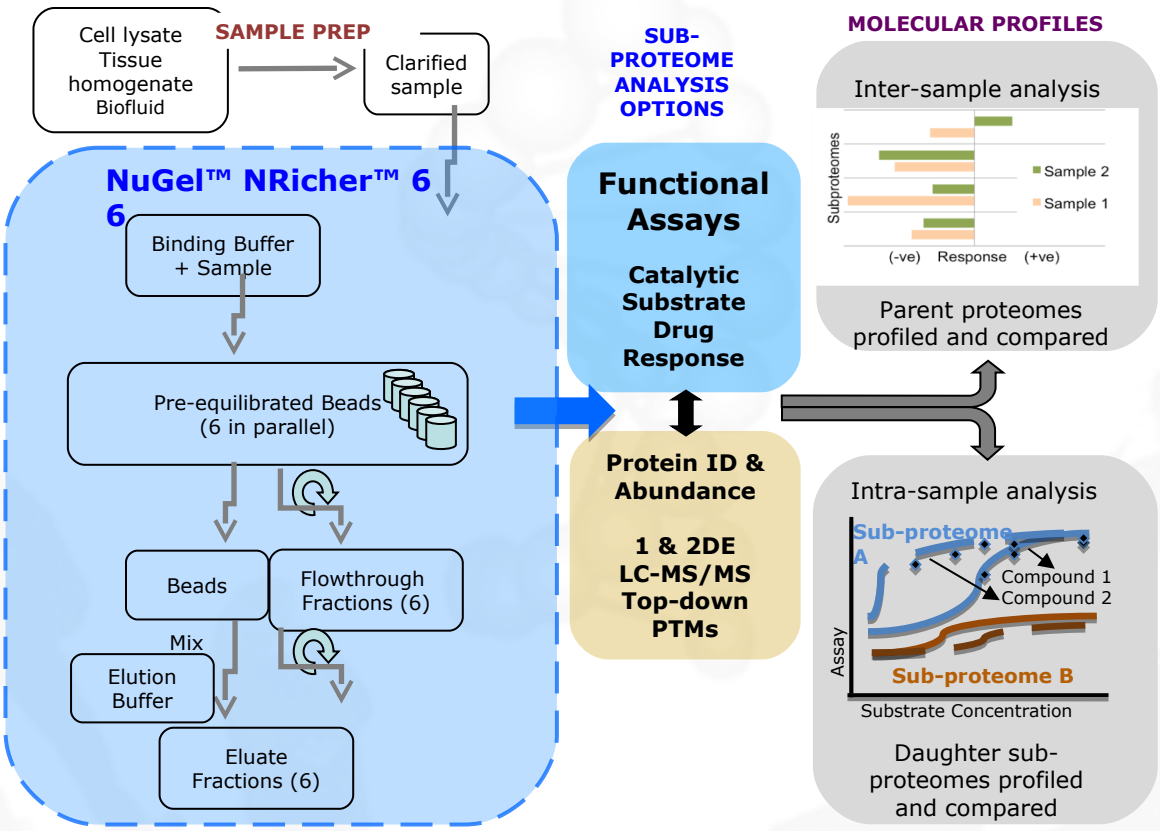

The **NuGel™ NRicher™ 6** product kit includes 6 mixed mode bead surfaces, binding and elution buffers and associated separations protocols. Each prep processes approximately 0.5-1.0 mg total protein, and produces 12 daughter sub-proteomes in 50 µl volumes in less than 1 hour.

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 6.0	15 ml	75 ml
***PRO*-EB** Elution Buffer, pH 10.0	1.5 ml	7.5 ml
Spin-X microfuge filters	30	150

Refrigerate upon arrival.

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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. The sample should be clarified and free of insoluble or colloidal cellular debris. The ideal pH for samples should be around 6-7.

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 [5,000-7,000]xg for 4 min.

1) Add 25 µl of ***PRO*-BB binding buffer** and 25 µl of "delipidated 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 50 µl of ***PRO*-BB 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 50 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 10.0, 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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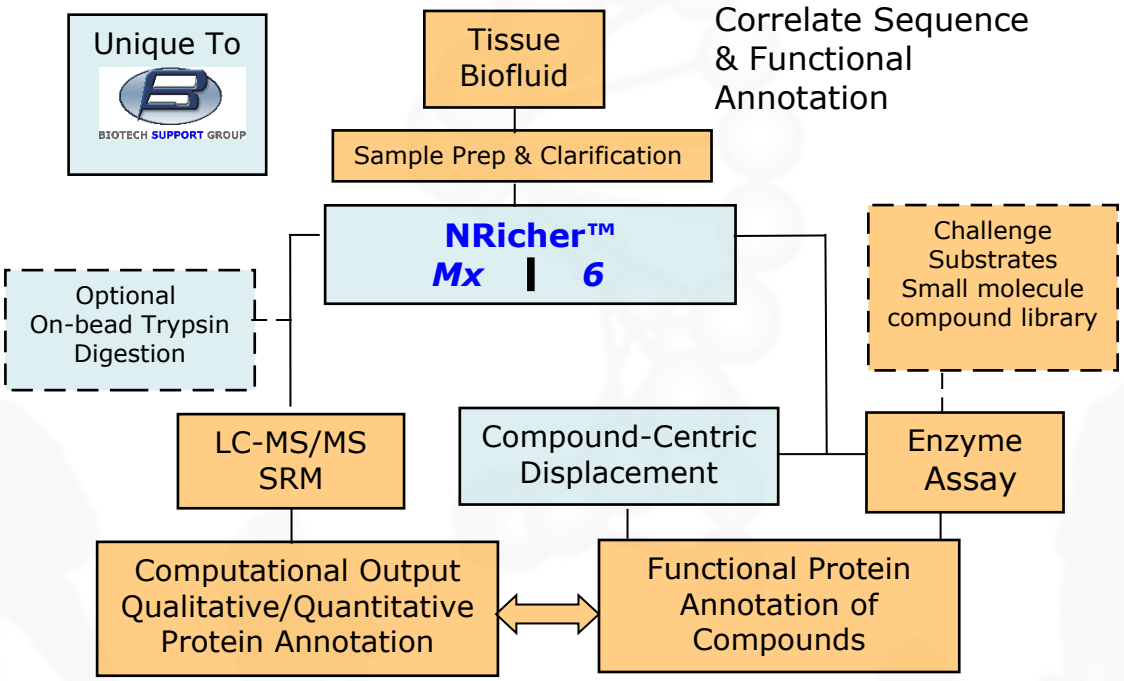
NuGel™-Based Functional Proteomics Products in Proteomic Workflows



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

For more information on functional and chemical proteomic applications, download our *Functional & Chemical Proteomics Handbook* at
http://biotechsupportgroup.com/sites/default/files/Biotech%20Support%20Group's%20Functional%20Proteomics%20Handbook%20030814%20v1

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References

Matthew P. Kuruc, Swapan Roy. The Functional & Chemical Proteomics Handbook 03/2014

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).

New Chemical Proteomic Methods To Access Drug-Protein Interactions

CONTACT US

We welcome your questions and comments regarding our products.

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