



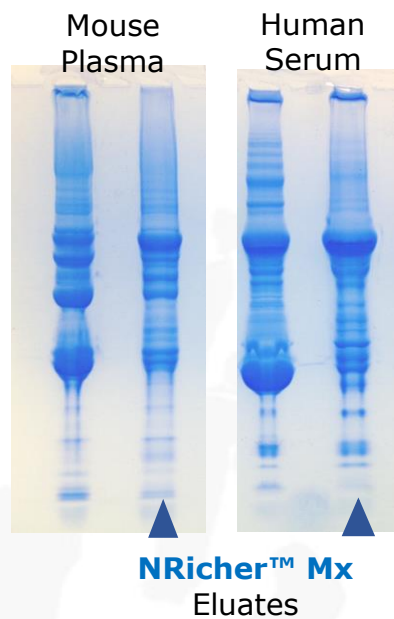
## BIOTECH SUPPORT GROUP

### 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
- ❖ Suitable for small volume mg scale preps

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 **NuGel™**-based **NRicher™** 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.





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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.

**NuGel™ NRicher™ Mx supports two application protocols:**

- **Compound Centric Displacement Proteomics (CCDP) &**
- **Low Abundance Proteome Enrichment**

**The protocols are supplied as two separate user documents.**

Kit Contains:	NuGel™ NRicher™ Mx 10	NuGel™ NRicher™ Mx 50
<b>NuGel™ NRicher™ Mx</b> beads	150 mg	750 mg
<b>PRO-BB</b> Binding Buffer, pH 6	3 ml	15 ml
<b>PRO-WB</b> Wash Buffer pH 7	5 ml	25 ml
<b>PRO-CEB</b> Elution Buffer, pH 7 (for <b>Compound Centric Displacement Proteomics Protocol</b> ) only	1 ml	5 ml
<b>PRO-EB</b> Elution Buffer, pH 10 (for <b>Low Abundance Proteome Enrichment Protocol</b> ) only	1 ml	5 ml
Spin-X microfuge filters	10	50

**\*Refrigerate upon arrival.**



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### Low Abundance Proteome Enrichment

#### Protocol

**Step 1 - Sample Preparation:** The protocol is based on 100  $\mu$ l 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  $\mu$ l be applied.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45  $\mu$ m 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  $\mu$ l 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.

**For Steps 3 & 4.** All centrifugations are for 4 minutes at 10,000 rpm.

#### **Step 3 - Separations.**

- 1) Add 100  $\mu$ l of **PRO-BB binding buffer** and 100  $\mu$ l (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  $\mu$ l 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  $\mu$ l **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

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 **NuGel™ NRicher™ 6** and **NuGel™ NRicher™ Mx** support functional and chemical proteomics and can:

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

**Please consult our Functional & Chemical Proteomics Handbook online, to see how NuGel™ NRicher™ Mx and NuGel™ NRicher™ 6 complement each other.**

### 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](#)

**US HUPO 2014. Frontiers in Proteomics: Advancing Biology through Technology and Computation.**

[NuGel™ PROfessor™](#) abstract entitled "[Compound-Centric Displacement Proteomics - An Advantaged Method To Survey Small Molecule-Protein Interactions](#)" poster board 096 presented at US HUPO 2014

### CONTACT US

**We welcome your questions and comments regarding our products.**

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