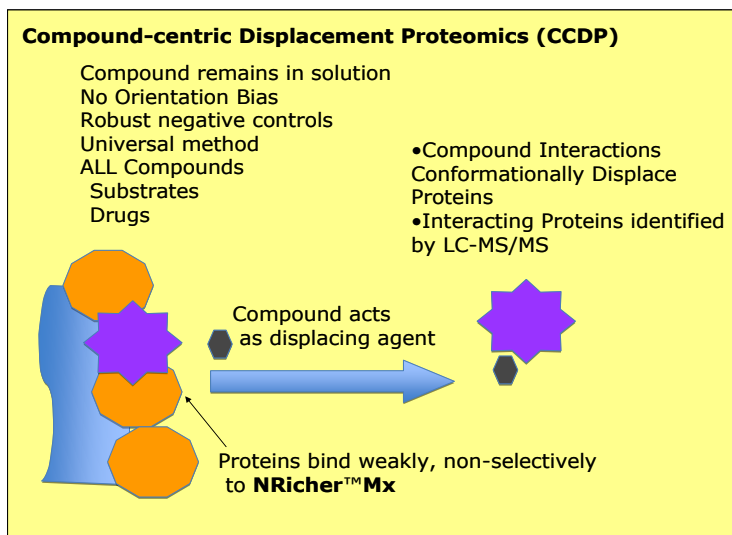




BIOTECH SUPPORT GROUP

NuGel™ NRicher™ Mx Compound Centric Displacement Proteomics (CCDP)

- ❖ Non-covalently binds proteins, a subset of which can be displaced upon introduction of soluble small compounds
- ❖ Coupled to LC-MS, spectral counts of affinity-eluted pools can serve as quantitative metrics to characterize and identify interacting proteins
- ❖ Gain efficiencies over prior covalent-based substitution methods: more complete survey, no orientation bias, robust background controls
- ❖ Applications in drug target deconvolution, on-target/off-target specificity, and personalized medicine.



MS2 Spectral Counts of CCDP-derived Sub-proteomes

Protein Description	Caffeine	Imatinib	Neg.Cont.
Hemoglobin subunit beta-1	87	550	53
Glucose-6-phosphate isomerase	192	459	76
Malate dehydrogenase	117	356	35
Transketolase	72	160	24
Cytochrome c, somatic	47	123	3
Succinyl-CoA:3-ketoacid transf	69	122	19
Transgelin	0	84	0
Annexin A2	26	66	0
Fumarate hydratase	17	42	2
Annexin A3	5	36	0
Glutathione reductase	9	38	0

A partial list of LC-MS/MS identification and spectral counts demonstrate Imatinib interaction proteins from



BIOTECH SUPPORT GROUP

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
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-CEB Elution Buffer, pH 7 (for Compound Centric Displacement Proteomics Protocol) only	1 ml	5 ml
PRO-EB Elution Buffer, pH 10 (for Low Abundance Proteome Enrichment Protocol) only	1 ml	5 ml
Spin-X microfuge filters	10	50



BIOTECH SUPPORT GROUP

Compound Centric Displacement Proteomics (CCDP)

Protocol

Step 1 - Sample Preparation: The protocol is based on 100 μ 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 μ l be applied.

For best results – the lysate should be clear and free of colloidal material. We recommend first filtering through a 0.45 μ 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 μ 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 μ l of **PRO-BB binding buffer** and 100 μ 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 μ 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. Repeat wash step 2 again.

Step 4 – Compound-centric displacement elution.

For compound-centric displacement proteomic, any challenging displacement compound can be added into the **PRO-CEB Elution buffer** (subject to solubility constraints) and used as an eluate. Use 60 μ l. Mix to homogeneously resuspend. Shake the sample for 10 minutes. Centrifuge. Collect eluate filtrates for analyses.



BIOTECH SUPPORT GROUP

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.

Tel: 732-274-2866, 800-935-0628 Mon – Fri 9am-6pm EST.

Email: sales@biotechsupportgroup.com

Mail: 1 Deer Park Drive, Suite M, Monmouth Junction, NJ 08852

Web: www.biotechsupportgroup.com