Sample Preparation



ProteoMiner™ Protein Enrichment Technology

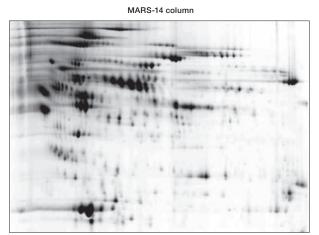
Digging Deeper in the Proteome



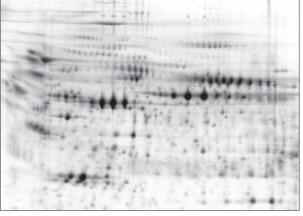


ProteoMiner Technology vs. an Agilent MARS-14 Column

A side-by-side comparison of the ProteoMiner protein enrichment kit and the Agilent MARS-14 column was performed using a plasma sample. Following treatment with the ProteoMiner beads or the use of a MARS column, the samples were analyzed by two-dimensional (2-D) gel electrophoresis. The sample treated with the ProteoMiner beads showed many more protein spots than the sample depleted with the MARS-14 column. These results suggest that the ProteoMiner kit is a superior product for sample preparation prior to low-abundance protein studies.



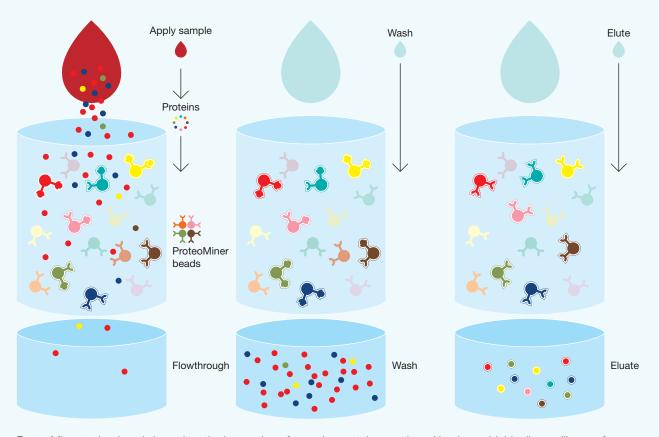




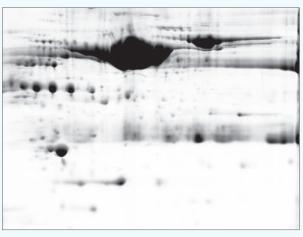
Results of comparison of two protein sample preparation methods. 2-D gel electrophoresis was performed on a plasma sample depleted with an Agilent MARS-14 column (left) and with the ProteoMiner kit (right). A substantially greater number of proteins were detected after treatment with the ProteoMiner kit. Data provided by AstraZeneca.

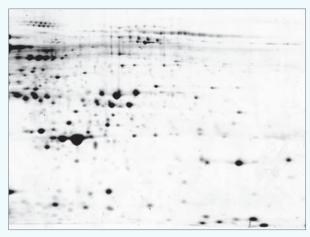
ProteoMiner Protein Enrichment Kits Uncover Low-Abundance Proteins

ProteoMiner protein enrichment technology is a novel sample preparation tool used to compress the dynamic range of protein concentrations in complex biological samples. The presence of high-abundance proteins in complex biological samples (for example, albumin and IgG in serum or plasma) makes the detection of medium- and low-abundance proteins extremely challenging. ProteoMiner protein enrichment kits provide a method for overcoming this challenge, allowing the exploration of the entire proteome.



ProteoMiner technology is based on the interaction of complex protein samples with a large, highly diverse library of hexapeptides bound to chromatographic supports. In theory, each unique hexapeptide binds to a unique protein sequence. Because the bead capacity limits binding capacity, high-abundance proteins quickly saturate their ligands (red and yellow beads) and excess protein is washed out during the procedure. In contrast, low-abundance proteins are concentrated on their specific ligands (pink and teal beads), thereby decreasing the dynamic range of proteins in the sample. When analyzed in downstream applications, the number of proteins detected is dramatically increased.





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The ProteoMiner protein enrichment kit improves resolution and spot counts in 2-D gels. In an untreated sample, albumin and other high-abundance proteins dominate the gel, obscuring signals from less abundant proteins; on a gel generated using an equal amount of total protein from a treated serum sample, however, resolution is dramatically improved and a greater number of protein spots are visualized.

Key Benefits of ProteoMiner Technology

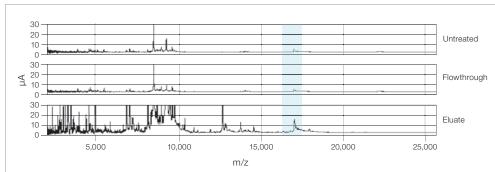
- Decreases the amount of high-abundance proteins without immunodepletion; prevents co-depletion of proteins bound to highabundance proteins
- Enriches and concentrates low-abundance proteins that cannot be detected through traditional methods
- Compresses the dynamic range of protein concentration in a variety of samples and is not dependent on a predefined set of antibodies as are immunodepletion products
- Can be used for differential expression analysis
- Is compatible with all major downstream protein analysis techniques

Convenient Format

- Spin column format is easy to use
- One time use avoids potential for contamination from carryover between samples
- All necessary columns and reagents provided in kits

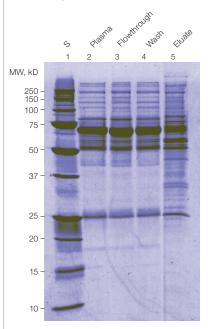
Discover More Proteins

Whether you use 1-D or 2-D gel electrophoresis, chromatography, surface-enhanced laser desorption/ionization (SELDI), or other mass spectrometry technologies for your downstream analysis, ProteoMiner kits provide a tool for digging deeper in the proteome.

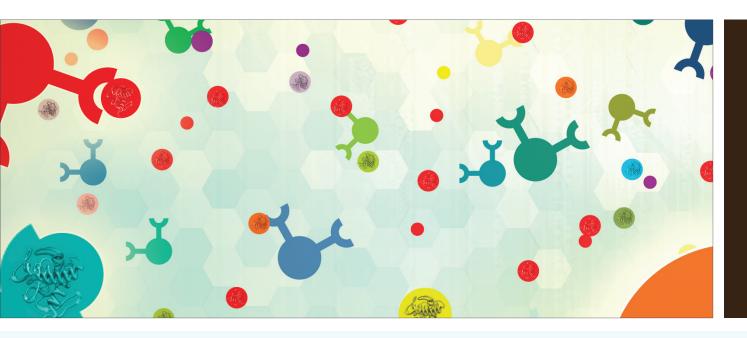


The ProteoMiner protein enrichment kit improves peak counts in SELDI analysis.

ProteinChip® SELDI system analysis with CM10 array of untreated plasma reveals 170 peaks, while plasma treated with the ProteoMiner protein enrichment kit (eluate) yields 263 peaks, or 55% more. One peak (shaded) was selected to show the enrichment experienced after the sample was treated with the ProteoMiner beads.



SDS-PAGE analysis of plasma treated with ProteoMiner protein enrichment beads. A plasma sample (1 ml) was treated with ProteoMiner protein enrichment beads, and 75 µg protein each from the original sample (lane 2), flowthrough (lane 3), wash (lane 4), and elution fractions was analyzed by SDS-PAGE. S, Precision Plus Protein™ standard.



Enhance Potential for Biomarker Discovery With ProteoMiner Technology

In biomarker discovery studies, accurate quantitation of low-abundance proteins in both disease and control samples is critical. ProteoMiner technology preserves quantitative information and enriches low-abundance proteins to make biomarker discovery easier and more likely.

Untreated 0 ng/µl 1 ng/µl 5 ng/µl 10 ng/μl 0 0 0 0 20 ng/µl 40 ng/µl 80 ng/µl 160 ng/µl Treated 0 ng/µl 1 ng/µl 5 ng/µl 10 ng/µl

ProteoMiner technology enriches proteins while maintaining the relative expression levels of low- to medium-abundance proteins. A series of plasma samples were spiked with different concentrations of serum amyloid A (SAA), left untreated (top) or treated with the ProteoMiner protein enrichment beads (bottom), and analyzed by 2-D gel electrophoresis. Relative intensities of the SAA spots (circled) are proportional to the amount of SAA added both before and after treatment with the ProteoMiner beads. The SAA spots are detected at a lower concentration (~5 ng/µl) in samples treated with ProteoMiner beads compared to untreated ones (~10 ng/µl), indicating that the ProteoMiner kit successfully enriched SAA.

80 ng/µl

40 ng/µl

Multiple Kits Available

To provide flexibility for use with multiple downstream applications, multiple formats are available — including small- and large-capacity kits optimized for varying protein load amounts. These kits have been optimized for use with serum and plasma samples, but they may be used with other sample types with at least 10 mg (with small-capacity kit) or 50 mg (with large-capacity kit) total protein. When used with the recommended amount of protein, the approximate yield is approximately 170 µg (for small-capacity kits) and 1 mg (for large-capacity kits). Both small- and large-capacity kits are available in the standard single elution format or the sequential elution format.

ProteoMiner Protein Enrichment Kits

ProteoMiner protein enrichment kits are available in both small- and large- capacity formats and provide columns and all necessary reagents for accessing low-abundance proteins in a variety of biological samples. The kits are compatible with the majority of downstream proteomics applications and can process up to ten samples. Introductory kits can process two samples.

ProteoMiner protein enrichment kits:

- Utilize single elution reagent in simple, easy-to-perform process
- Provide maximum compatibility with downstream applications

ProteoMiner Sequential Elution Kits

ProteoMiner sequential elution kits are available in both small- and large- capacity format and are designed for researchers who wish to elute their proteins into multiple fractions to detect additional proteins. These kits have been optimized for SELDI experiments and provide columns and reagents for processing up to ten samples (reagents are not compatible for direct use with 2-D gel electrophoresis).

ProteoMiner sequential elution kits:

- Use multiple elution reagents to sequentially elute proteins based on different properties
- Fractionate proteins to improve detection and resolution of proteins for SELDI analysis

For more information, request bulletin 5632 or visit us on the Web at **www.bio-rad.com/proteominer/**.

Bibliography

Guerrier L et al. (2006). Reducing protein concentration range of biological samples using solid-phase ligand libraries. J Chromatogr B Analyt Technol Biomed Life Sci 833, 33-40.

Sennels L et al. (2007). Proteomic analysis of human blood serum using peptide library beads. J Proteome Res 6, 4055-4062.

Thulasiraman V et al. (2005). Reduction of the concentration difference of proteins in biological liquids using a library of combinatorial ligands. Electrophoresis 26, 3561-3571.

Ordering Information

Catalog # Description

ProteoMiner Protein Enrichment Kits

163-3006	ProteoMiner Protein Enrichment Small-Capacity Kit, for processing 10 mg of sample, 10 preps, includes 10 spin columns, wash buffer, elution reagents, collection tubes. Yields ~170 µg protein
163-3007	ProteoMiner Protein Enrichment Large-Capacity Kit, for processing 50 mg of sample, 10 preps, includes 10 spin columns, wash buffer, elution reagents, collection tubes. Yields ~1 mg protein
163-3008	ProteoMiner Protein Enrichment Introductory Small-Capacity Kit, for processing 10 mg of sample, 2 preps, includes 2 spin columns, wash buffer, elution reagents, collection tubes. Yields ~170 µg protein
163-3009	ProteoMiner Protein Enrichment Introductory Large-Capacity Kit, for processing 50 mg of sample, 2 preps, includes 2 spin columns, wash buffer, elution

ProteoMiner Sequential Elution Kits

163-3010	ProteoMiner Sequential Elution Small-Capacity Kit, for processing 10 mg of sample, 10 preps, includes 10 spin columns, wash buffer, 4 sequential elution reagents, collection tubes. Yields ~180 μg protein in total, divided across 4 fractions
163-3011	ProteoMiner Sequential Elution Large-Capacity Kit, for processing 50 mg of sample, 10 preps, includes 10 spin columns, wash buffer, 4 sequential elution reagents, collection tubes. Yields ~570 μg protein in total, dvided across 4 fractions

reagents, collection tubes. Yields ~1 mg protein

ProteoMiner Kit Accessories

163-3003 **ProteoMiner Sequential Elution Reagents**, 10 preps, includes reagents only (columns not included), to be

used with 163-3006 or 163-3007

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The SELDI process is covered by US patents 5,719,060, 6,225,047, 6,579,719, and 6,818,411 and other issued patents and pending applications in the US and other jurisdictions.



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