
ProteoMiner™ Protein Enrichment Kits

Instruction Manual

Catalog #

163-3003

163-3006

163-3007

163-3008

163-3009

163-3010

163-3011

163-3012

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Section 1

Introduction

The ProteoMiner™ technology is a novel sample preparation tool used for the compression of the dynamic range of the protein concentration in complex biological samples. High-abundance proteins present in complex biological samples like sera or plasma, make the detection of medium- and low-abundance proteins extremely challenging. This technology provides a method of overcoming this challenge, allowing for the exploration of the entire proteome.

This is accomplished through the use of a large, highly diverse bead-based library of combinatorial peptide ligands. When complex biological samples are applied to the beads, the high-abundance proteins saturate their high affinity ligands and excess protein is washed away. In contrast, the medium- and low-abundance proteins are concentrated on their specific affinity ligands. This reduces the dynamic range of protein concentrations while maintaining representatives of all proteins within the original sample.

Section 2

Kit Specifications

ProteoMiner™ Small-Capacity Kit (Catalog #163-3006)

Provides reagents for processing 10 samples. Compatible with 2-D gel electrophoresis and other downstream protein separation analysis methods.

- Spin Columns. 10 columns each containing 500 µl bead slurry (4% beads, 20% v/v aqueous EtOH), 20 µl settled bead volume
- Wash Buffer. 50 ml PBS buffer (150 mM NaCl, 10 mM NaH₂PO₄, pH 7.4)
- Elution Reagent. 2 vials, lyophilized urea CHAPS (8 M urea, 2% CHAPS)
- Rehydration Reagent. 5 ml, 5% acetic acid
- Collection Tubes. 20 capless, 2 ml centrifuge tubes; 10 capped, 2 ml centrifuge tubes
- Instruction Manual
- Quick Start Guide

ProteoMiner Large-Capacity Kit (Catalog #163-3007)

Provides reagents for processing 10 samples. Compatible with 2-D gel electrophoresis and other downstream protein separation analysis methods.

- Spin Columns. 10 columns each containing 500 µl bead slurry (20% beads, 20% v/v aqueous EtOH), 100 µl settled bead volume

- Wash Buffer. 50 ml PBS buffer (150 mM NaCl, 10 mM NaH₂PO₄, pH 7.4)
- Elution Reagent. 5 vials, lyophilized urea CHAPS (8 M urea, 2% CHAPS)
- Rehydration Reagent. 5 ml, 5% acetic acid
- Collection Tubes. 20 capless, 2 ml centrifuge tubes; 10 capped, 2 ml centrifuge tubes
- Instruction Manual
- Quick Start Guide

ProteoMiner Introductory Small-Capacity Kit (Catalog #163-3008)

Contains two spin columns, all other reagents are the same as in the ProteoMiner small-capacity kit above. Introductory kit provides reagents for processing two samples.

ProteoMiner Introductory Large-Capacity Kit (Catalog #163-3009)

Contains two spin columns, all other reagents are the same as in the ProteoMiner large-capacity kit above. Introductory kit provides reagents for processing two samples.

ProteoMiner Sequential Elution Small-Capacity Kit (Catalog #163-3010)

This kit combines the ProteoMiner small-capacity kit (catalog #163-3006) and the ProteoMiner sequential elution reagents (catalog #163-3003) and is designed to provide multiple elution options for researchers using SELDI or other downstream protein separation analysis methods, and who wish to access additional proteins. This kit is NOT compatible with 2-D gel electrophoresis.

ProteoMiner Sequential Elution Large-Capacity Kit (Catalog #163-3011)

This kit combines the ProteoMiner large-capacity kit (catalog #163-3007) and the ProteoMiner sequential elution reagents (catalog #163-3003) and is designed to provide multiple elution options for researchers using SELDI or other downstream protein separation analysis methods, and who wish to access additional proteins. This kit is NOT compatible with 2-D gel electrophoresis.

ProteoMiner Sequential Elution Reagents (Catalog #163-3003)

To be used in combination with the ProteoMiner kits (catalog #s163-3006 or 163-3007), this product provides reagents for processing 10 samples. The sequential elution reagents are available for researchers using SELDI or other downstream protein separation analysis methods, and who wish to access

additional proteins. These reagents are NOT compatible with 2-D gel electrophoresis.

- Elution Reagent 1. 5 ml 1 M sodium chloride, 20 mM HEPES, pH 7.4
- Elution Reagent 2. 5 ml 200 mM glycine, pH 2.4
- Elution Reagent 3. 5 ml 60% ethylene glycol in water
- Elution Reagent 4. 5 ml 33.3% 2-propanol, 16.7% acetonitrile, 0.1% trifluoroacetic acid
- Plasma Preparation Buffer. 1.5 ml 1 M sodium citrate, 20 mM HEPES, pH 7.4
- Collection tubes. 30 capped, 2 ml centrifuge tubes

Items required but not provided:

- Microcentrifuge or vacuum manifold (available through Bio-Rad, catalog #732-6470)
- Pipet
- Proteomics grade water (available through Bio-Rad, catalog #163-2091)

ProteoMiner Beads (Catalog #163-3012)

Provides 525 mg of dry beads, columns and reagents not provided.

Section 3

Storage Conditions

Store the unopened kit at 4°C. After reconstituting lyophilized elution reagent store any remaining material at -20°C for up to one week.

Section 4

Reagent Preparation

Prepare elution reagent by adding 610 μ l rehydration reagent to one vial lyophilized elution reagent. Following rehydration, each vial will contain enough elution reagent for processing two samples. If preparing an uneven number of columns, you will have remaining material that will be required for subsequent preparations. Remaining material may be stored at -20°C for up to one week. However, it is recommended to make this solution fresh each time you use the kit.

Section 5

Sample Considerations

This kit has been optimized for plasma (nonheparinized) and serum samples. Sample loading and buffer compatibility have not been validated for other sample types. However, ProteoMiner™ technology has successfully been applied to other sample types including urine (Righetti et al. 2005), bile (Housset et al. 2007), platelets (Boschetti et al. 2008), red blood cell extract (Boschetti et al. 2008), and egg white extract (Boschetti et al. 2008).

Results with other sample types will vary depending on the amount of protein in your sample; best results are obtained with 50 mg protein for the large-capacity kits and 10 mg for the small-capacity kits. **Caution: When working with human plasma/serum it is important to follow biohazardous material handling guidelines.**

The ratio of protein to beads is crucial for optimal performance of ProteoMiner kits. The dynamic range of the protein concentration in the sample is reduced when the high-abundant proteins saturate their ligands and the low-abundant proteins bind to a sufficient number of ligands to allow for enrichment. Therefore, in order to achieve optimal results, it is important to load the recommended amount of protein.

Section 6

Instructions for Use With ProteoMiner™ Large-Capacity Kits (Catalog #s 163-3007 and 163-3009)

This protocol has been **optimized for plasma and serum** samples with protein concentrations of ≥ 50 mg/ml (requires total protein load ≥ 50 mg). **For other sample types, please refer to Section 5: Sample Considerations.**

Note: A ProteoMiner sequential elution kit (catalog #163-3011) is available for researchers using SELDI or other downstream protein separation analysis methods, other than 2-D gel electrophoresis, and who wish to access additional proteins.

If using the ProteoMiner sequential elution kit, refer to page 9.

Step 1 – Column Preparation

Vacuum (at 16 mm Hg) can replace centrifugation for column preparation, sample binding and sample wash steps if desired. (Vacuum manifold is available through Bio-Rad, catalog #732-6470.)

1. First remove the top cap and then snap off the bottom cap from each of the spin columns you will be using.

Note: Do not discard top or bottom caps, they will be reused throughout the protocol. If beads settle in top cap, replace after removing bottom plug and centrifuge with top cap on column. To use bottom cap as a plug, invert and firmly place in bottom of spin column.

2. Place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the storage solution. Discard collected material.

Note: Kit contains one capless collection tube per spin column for the following steps: column preparation, sample binding, and sample wash. Kit contains one capped collection tube per spin column to be used for the elution step, allowing for easy storage of your eluted sample.

3. Replace the bottom cap and add 600 µl wash buffer, then replace top cap.
4. Rotate column end-to-end several times over a 5 min period.
5. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove buffer. Discard collected material.
6. Repeat steps 3 and 4.
7. Remove caps, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the wash buffer. Discard collected material.
8. Replace bottom cap on spin column. The column now contains 100 µl of settled beads and is ready for sample binding.

Step 2 – Sample Binding

Samples should be free of precipitate. If needed, centrifuge samples at 10,000 x g for 10 min to clarify. Take precautions to avoid the bottom aggregate proteins and top lipid layer when recovering your sample. It is recommended that at least 1 ml of sample (protein concentration ≥ 50 mg/ml) is added to the column, as lower volumes may not achieve optimal results. **For other sample types, please refer to Section 5: Sample Considerations.**

1. Add 1 ml of sample to column. Replace top cap and rotate column on a platform or rotational shaker for 2 hr at room temperature.

Note: If using plasma, clumping may occur after 1 hr of binding; this is expected and will not negatively impact your sample preparation. Heparinized plasma is not compatible with this kit.

Step 3 – Sample Wash

1. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
2. Replace the bottom cap and add 600 µl of wash buffer to column. Replace top cap and rotate from end-to-end several times over a 5 min period.
3. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
4. Repeat steps 2 and 3 three more times.

Step 4 – Elution

1. After all wash buffer has been removed, replace the bottom cap and add 600 µl deionized water.
2. Attach top cap and rotate end-to-end for 1 min.
3. Remove caps, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove water. Discard collected material.

If using vacuum up to this point, you will now need to switch to centrifugation.

4. Attach bottom cap to the column (take caution to ensure the bottom cap is tightly attached). Add 100 µl of rehydrated elution reagent (refer to Section 4 for rehydration instructions) to the column and replace top cap. Lightly vortex for 5 sec.

Note: For 2-D users who plan to use DIGE, this elution reagent will require clean up and pH adjustment (described in Section 11). As an alternative you may elute with DIGE buffer. However, this may result in a decreased yield and number of protein spots.

5. Incubate column at room temperature, lightly vortex several times over a period of 15 min.
6. Remove caps, place in a clean collection tube labeled E1 and centrifuge at 1,000 x g for 30–60 sec. This elution contains your eluted proteins; do not discard.
7. Repeat steps 5–6 two more times. (Elutions may be pooled or analyzed individually. If analyzed individually, you will need additional collection tubes not provided with kit.)
8. Store elution at –20°C or proceed with downstream analysis. (For best results, we recommend a clean up of your sample prior to analysis. See Section 11 for more information on preparing sample for analysis.)

Section 7

Instructions for Use With ProteoMiner™ Small-Capacity Kits (Catalog #s 163-3006 and 163-3008)

This protocol has been **optimized for plasma and serum** samples with protein concentrations of ≥ 50 mg/ml (requires total protein load ≥ 10 mg). **For other sample types, please refer to Section 5: Sample Considerations.**

Note: A ProteoMiner sequential elution kit (catalog #163-3010) is available for researchers using SELDI or other downstream protein separation analysis methods, other than 2-D gel electrophoresis, and who wish to access additional proteins.

If using the ProteoMiner sequential elution kit, refer to page 12.

Step 1 – Column Preparation

Vacuum (at 16 mm Hg) can replace centrifugation for column preparation, sample binding, and sample wash steps if desired. (Vacuum manifold is available through Bio-Rad, catalog #732-6470.)

1. First remove the top cap and then snap off the bottom cap from each of the spin columns you will be using.

Note: Do not discard top or bottom caps, they will be reused throughout the protocol. If beads settle in top cap, replace after removing bottom plug and centrifuge with top cap on column. To use bottom cap as a plug, invert and firmly place in bottom of spin column.

2. Place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the storage solution. Discard collected material.

Note: Kit contains one capless collection tube per spin column for the following steps: column preparation, sample binding, and sample wash. Kit contains one capped collection tube per spin column to be used for the elution step, allowing for easy storage of your eluted sample.

3. Replace the bottom cap and add 200 μ l wash buffer, then replace top cap.
4. Rotate column end-to-end several times over a 5 min period.
5. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove buffer. Discard collected material.
6. Repeat steps 3 and 4.
7. Remove caps, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the wash buffer. Discard collected material.

8. Replace bottom cap on spin column. The column now contains 20 μ l of settled beads and is ready for sample binding.

Step 2 – Sample Binding

Samples should be free of precipitate. If needed, centrifuge samples at 10,000 x g for 10 min to clarify. Take precautions to avoid the bottom aggregate proteins and top lipid layer when recovering your sample. It is recommended that at least 200 μ l of sample (protein concentration $\geq$ 50 mg/ml) is added to the column, as lower volumes may not achieve optimal results. **For other sample types, please refer to Section 5: Sample Considerations.**

1. Add 200 μ l of sample to column. Replace top cap and rotate column on a platform or rotational shaker for 2 hr at room temperature.

Note: If using plasma, clumping may occur after 1 hr of binding; this is expected and will not negatively impact your sample preparation. Heparinized plasma is not compatible with this kit.

Step 3 – Sample Wash

1. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
2. Replace the bottom cap and add 200 μ l of wash buffer to column. Replace top cap and rotate from end-to-end several times over a 5 min period.
3. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
4. Repeat steps 2 and 3 two more times.

Step 4 – Elution

1. After all wash buffer has been removed, replace the bottom cap and add 200 μ l deionized water.
2. Attach top cap and rotate end-to-end for 1 min.
3. Remove caps, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove water. Discard collected material.
If using vacuum up to this point, you will now need to switch to centrifugation.
4. Attach bottom cap to the column (take caution to ensure the bottom cap is tightly attached). Add 20 μ l of rehydrated elution reagent (refer to Section 4 for rehydration instructions) to the column and replace top cap. Lightly vortex for 5 sec.

Note: For 2-D users who plan to use DIGE, this elution reagent will require clean up and pH adjustment (described in Section 11). As an alternative you may elute with DIGE buffer; however, this may result in a decreased yield and number of protein spots.

5. Incubate column at room temperature, lightly vortex several times over a period of 15 min.
6. Remove caps, place in a clean collection tube labeled E1 and centrifuge at 1,000 x g for 30–60 sec. This elution contains your eluted proteins. Do not discard.
7. Repeat steps 5–6 two more times. (Elutions may be pooled or analyzed individually. If analyzed individually, you will need additional collection tubes not provided with kit.)
8. Store elution at –20°C or proceed with downstream analysis. (For best results, we recommend a clean up of your sample prior to analysis. See Section 11 for more information on preparing sample for analysis.)

Section 8

Instructions for Use With Sequential Elution Kit (Large-Capacity)

The ProteoMiner™ Sequential Elution large-capacity kit combines the ProteoMiner kit (catalog #163-3007) and the ProteoMiner sequential elution reagents (catalog #163-3003) and is available for researchers using SELDI or other downstream protein separation analysis methods who wish to access additional proteins. This kit is NOT compatible with 2-D gel electrophoresis.

This protocol has been **optimized for plasma and serum** samples with protein concentration of ≥ 50 mg/ml (requires total protein load ≥ 50 mg). **For other sample types, please refer to Section 5: Sample Considerations.**

Step 1 – Column Preparation

(Reagents and columns required for this step are included in the ProteoMiner kit, catalog #163-3007.)

Vacuum (at 16 mm Hg) can replace centrifugation for column preparation, sample binding and sample wash steps if desired. (Vacuum manifold is available through Bio-Rad, catalog #732-6470.)

1. First remove the top cap and then snap off the bottom cap from each of the spin columns you will be using.

Note: Do not discard top or bottom caps, they will be reused throughout the protocol. If beads settle in top cap, replace after removing bottom plug and centrifuge with top cap on column. To use bottom cap as a plug, invert and firmly place in bottom of spin column.

2. Place the column in a capless collection tube and centrifuge at 1,000 x g

for 30–60 sec to remove the storage solution. Discard collected material.

Note: Kit contains one capless collection tube per spin column for the following steps: column preparation, sample binding, and sample wash. Kit contains one capped collection tube per spin column to be used for the elution step, allowing for easy storage of your eluted sample.

3. Replace the bottom cap and add 600 μ l wash buffer, then replace top cap.
4. Rotate column end-to-end several times over a 5 min period.
5. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove water. Discard collected material.
6. Repeat steps 3 and 4.
7. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the wash buffer. Discard collected material.
8. Replace bottom cap on spin column. The column is now ready for sample binding.

Step 2 – Sample Binding

Samples should be free of precipitate. Centrifuge samples at 10,000 x g for 10 min to clarify. Take precautions to avoid the bottom aggregate proteins and top lipid layer when recovering your sample. It is recommended that at least 1 ml of sample (protein concentration ≥ 50 mg/ml) is added to the column, lower volumes may not achieve optimal results. **For other sample types, please refer to Section 5: Sample Considerations.**

If using plasma: Add 900 μ l plasma (nonheparinized) and 100 μ l plasma preparation buffer to spin column. Replace top cap and rotate column on a platform or rotational shaker for 2 hr at room temperature. After approximately 1 hr of binding you may see clumping; this is expected and will not negatively impact your sample preparation. Heparinized plasma is not compatible with this kit.

For all other sample types: Add 1 ml of sample to column and replace top cap. Rotate column on a platform or rotational shaker for 2 hr at room temperature.

Step 3 – Sample Wash

(Reagents required for this step are included in the ProteoMiner kit, catalog #163-3007.)

1. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material. Centrifuge again at 1,000 x g for 30–60 sec to remove any remaining material. Discard collected material.

2. Replace the bottom cap and add 600 μ l of wash buffer to column, then replace top cap and rotate end-to-end several times over a 5 min period.
3. Remove bottom cap, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
4. Repeat steps 2 and 3 three more times.

Step 4 – Sequential Elution

(Reagents required for this step are included with the ProteoMiner sequential elution reagents, catalog #163-3003.)

When using the ProteoMiner sequential elution kit, the elution reagent and rehydration reagent supplied with the ProteoMiner kit are not needed. Instead use elution reagents 1–4 supplied with the ProteoMiner sequential elution reagents (catalog #163-3003).

1. Carefully add 200 μ l wash buffer on all sides of the column to ensure none of the beads are stuck to the sides of the column.
2. Centrifuge at 1,000 x g for 30–60 sec. Discard collected material. *If using vacuum up to this point, you will now need to switch to centrifugation.*
3. Attach bottom cap to the column (take caution to ensure the bottom cap is tightly attached). Add 100 μ l of elution reagent 1 to spin column. Incubate at room temperature and lightly vortex several times over a period of 10 min.
4. Remove bottom cap, place column in collection tube labeled F1 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
5. Repeat steps 3 and 4 two more times and collect both elutions in tube F1.
6. Attach bottom cap to column and add 100 μ l of elution reagent 2 to column. Incubate at room temperature and lightly vortex several times over a period of 10 min.
7. Remove bottom cap, place column in collection tube labeled F2 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
8. Repeat steps 6 and 7 two more times and collect both eluents in tube F2. (If treating plasma samples, a white precipitate will form when adding elution reagent 2. Before using eluent in downstream applications, spin and use supernatant).
9. Attach bottom cap to column, place column in collection tube labeled F3 and add 100 μ l of elution reagent 3 to column. Incubate at room temperature, and lightly vortex several times over a period of 10 min.
10. Centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.

11. Repeat steps 9 and 10 two more times and collect both elutions in tube F3.
12. Attach bottom cap to column and add 100 μ l of elution reagent 4 to column. Incubate at room temperature and lightly vortex several times over a period of 5 min.
13. Remove bottom cap, place column in collection tube labeled F4 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
14. Repeat steps 12 and 13 two more times and collect both eluents in tube F4.
15. Store elutions at -20°C or proceed with downstream analysis. (See Section 9 for more information on preparing sample for analysis.)

Section 9

Instructions for Use With Sequential Elution Kit (Small-Capacity)

The ProteoMiner™ sequential elution small-capacity kit combines the ProteoMiner kit (catalog #163-3006) and the ProteoMiner sequential elution reagents (catalog #163-3003) and is available for researchers using SELDI or other downstream protein separation analysis methods who wish to access additional proteins. This kit is NOT compatible with 2-D gel electrophoresis.

This protocol has been **optimized for plasma and serum** samples with protein concentration of ≥ 50 mg/ml (requires total protein load ≥ 10 mg). **For other sample types, please refer to Section 5: Sample Considerations.**

Step 1 – Column Preparation

(Reagents and columns required for this step are included in the ProteoMiner kit, catalog #163-3006.)

Vacuum (at 16 mm Hg) can replace centrifugation for column preparation, sample binding, and sample wash steps if desired. (Vacuum manifold is available through Bio-Rad, catalog #732-6470.)

1. First remove the top cap and then snap off the bottom cap from each of the spin columns you will be using.

Note: Do not discard top or bottom caps, they will be reused throughout the protocol. If beads settle in top cap, replace after removing bottom plug and centrifuge with top cap on column. To use bottom cap as a plug, invert and firmly place in bottom of spin column.

2. Place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the storage solution. Discard collected material.

Note: Kit contains one capless collection tube per spin column for the following steps: column preparation, sample binding, and sample wash. Kit contains one capped collection tube per spin column to be used for the elution step, allowing for easy storage of your eluted sample.

3. Replace the bottom cap and add 200 μ l wash buffer, then replace top cap.
4. Rotate column end-to-end several times over a 5 min period.
5. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove water. Discard collected material.
6. Repeat steps 3 and 4.
7. Remove bottom cap, place the column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec to remove the wash buffer. Discard collected material.
8. Replace bottom cap on spin column. The column now contains 20 μ l of settled beads and is ready for sample binding.

Step 2 – Sample Binding

Samples should be free of precipitate. Centrifuge samples at 10,000 x g for 10 min to clarify. Take precautions to avoid the bottom aggregate proteins and top lipid layer when recovering your sample. It is recommended that at least 200 μ l of sample (protein concentration $\geq$ 50 mg/ml) is added to the column, lower volumes may not achieve optimal results. **For other sample types, please refer to Section 5: Sample Considerations.**

If using plasma: Add 180 μ l plasma (nonheparinized) and 20 μ l plasma preparation buffer to spin column. Replace top cap and rotate column end-to-end for 2 hr at room temperature. After approximately 1 hr of binding you may see clumping; this is expected and will not negatively impact your sample preparation. Heparinized plasma is not compatible with this kit.

For all other sample types: Add 200 μ l of sample to column and replace top cap. Rotate column on a platform or rotational shaker for 2 hr at room temperature.

Step 3 – Sample Wash

(Reagents required for this step are included in the ProteoMiner kit, catalog #163-3006.)

1. Remove caps, place column in a capless collection tube and centrifuge at 1,000 x g for 30–60 sec. Discard collected material.
2. Replace the bottom cap and add 200 μ l of wash buffer to column, then replace top cap and rotate end-to-end several times over a 5 min period.
3. Remove bottom cap, place column in a capless collection tube and

centrifuge at 1,000 x g for 30–60 sec. Discard collected material.

4. Repeat steps 2 and 3 two more times.

Step 4 – Sequential Elution

(Reagents required for this step are included with the ProteoMiner sequential elution reagents, catalog #163-3003.)

When using the ProteoMiner sequential elution kit, the elution reagent and rehydration reagent supplied with the ProteoMiner kit are not needed. Instead use elution reagents 1–4 supplied with the ProteoMiner sequential elution reagents (catalog #163-3003).

1. Carefully add 200 μ l wash buffer on all sides of the column to ensure none of the beads are stuck to the sides of the column.
2. Centrifuge at 1,000 x g for 30–60 sec. Discard collected material. If using vacuum up to this point, you will now need to switch to centrifugation.
3. Attach bottom cap to the column (take caution to ensure the bottom cap is tightly attached). Add 20 μ l of elution reagent 1 to spin column. Incubate at room temperature and lightly vortex several times over a period of 10 min.
4. Remove bottom cap, place column in collection tube labeled F1 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
5. Repeat steps 3 and 4 two more times and collect both elutions in tube F1.
6. Attach bottom cap to column and add 20 μ l of elution reagent 2 to column. Incubate at room temperature and lightly vortex several times over a period of 10 min.
7. Remove bottom cap, place column in collection tube labeled F2 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
8. Repeat steps 6 and 7 two more times and collect both eluents in tube F2. (If treating plasma samples, a white precipitate will form when adding elution reagent 2. Before using eluent in downstream applications, spin and use supernatant).
9. Attach bottom cap to column, place column in collection tube labeled F3 and add 20 μ l of elution reagent 3 to column. Incubate at room temperature, and lightly vortex several times over a period of 10 min.
10. Centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
11. Repeat steps 9 and 10 two more times and collect both elutions in tube F3.
12. Attach bottom cap to column and add 20 μ l of elution reagent 4 to column. Incubate at room temperature and lightly vortex several times over a period of 5 min.

13. Remove bottom cap, place column in collection tube labeled F4 and centrifuge at 1,000 x g for 30–60 sec to collect the elution. This elution contains your eluted proteins. Do not discard.
14. Repeat steps 12 and 13 two more times and collect both eluents in tube F4.
15. Store elutions at –20°C or proceed with downstream analysis. (See Section 11 for more information on preparing sample for analysis.)

Section 10

Instructions for Use With Bulk Beads

Step 1 – Preparation

1. Before using the ProteoMiner™ beads (525 mg), swell the beads by rehydrating with 10 ml, 20% v/v aqueous EtOH. Add the 20% EtOH swelling solution directly to the bottle and cap tightly.
2. Allow the beads to swell overnight for approximately 12 hrs at 4°C with gentle rocking or rotation.
3. The final slurry of the beads following rehydration will be approximately 20% ProteoMiner beads in 20% aqueous EtOH. However, it is recommended to measure and adjust the slurry to 20% beads before aliquoting into columns or plates in order to obtain the correct settled bead volumes provided in the table below. Carefully transfer the entire slurry to a sterile graduated container (for example a 15 ml screwcap conical tube). Cover the container and allow the beads to settle for approximately 30 min. After settling, adjust the volume of the swelling solution by carefully removing or adding additional swelling solution so that the ratio of settled bead volume to total slurry volume is 20% beads (for example, 2 ml of settled beads in 10 ml total slurry volume).
4. Store rehydrated beads at 4°C in a sealed container until ready for use.

Step 2 – Aliquoting Beads

1. Before aliquoting ProteoMiner beads into columns or plates, you must first calculate the appropriate amount of beads to use for your application. The ratio of protein to beads is crucial for optimal performance. The dynamic range of the protein concentration in the sample is reduced when the high-abundance proteins saturate their ligands and the low-abundance proteins bind to a sufficient number of ligands to allow for enrichment.

It is recommended to use a 10:1 ratio of sample volume (with protein concentration of ≥ 50 mg/ml) to settled bead volume. The following chart should help you determine what volume of beads you will need for your application.

Volume of sample available	Volume of 20% bead slurry required	Resulting settled bead volume (after swelling solution is removed)
200 µl	100 µl	20 µl
500 µl	250 µl	50 µl
1,000 µl	500 µl	100 µl

This chart assumes a protein concentration of ≥ 50 mg/ml, which is typical for serum. If your sample concentration is lower, adjust volumes accordingly to assure 0.5 mg of protein per µl of beads. It is not recommended to use less than 10 mg of protein.

- After determining the appropriate amount of beads to use for your application, carefully pipette the bead slurry into either a spin column (Bio-Rad catalog #732-6207) or 96-well plate with low-protein binding membrane (Available from Pall Corp., part number 5039). ***It is important to assure that the slurry is well mixed before pipetting to assure reproducibility of aliquoting. If beads have settled, lightly mix until resuspended.***

Step 3 – Prepare Reagents

- Prepare PBS wash buffer (150 mM NaCl, 10 mM NaH_2PO_4 , pH 7.4). Approximately 5 ml per sample will be required.
- Prepare elution reagent(s). See pages 1 and 2 for elution reagent options. Two elution protocols are available depending on your downstream application. It is recommended that the majority of users follow the single elution protocol that utilizes urea CHAPS/acetic acid (page 1). For SELDI users and others who wish to sequentially elute their proteins, we recommend the sequential elution protocol that utilizes four elution reagents (see page 2). The sequential elution reagents are also available for purchase (catalog #163-3003).

Step 4 – Sample Processing

- Depending on which settled bead volume and which set of elution reagents you have chosen, please refer to the appropriate protocol: single elution protocol, 100 µl settled bead volume (Section 6); single elution protocol, 20 µl settled bead volume (Section 7); sequential elution protocol, 100 µl settled bead volume (Section 8); sequential elution protocol, 20 µl settled bead volume (Section 9). These protocols are designed for use with 100 µl or 20 µl of settled beads. If using different amounts adjust accordingly. Additionally, if you choose to use a 96-well filter plate, vacuum will have to be used in place of centrifugation.
- After eluting your samples from the column/plate, refer to Section 11 for information on preparing your sample for analysis.

Section 11

Appendix

Preparation for Various Downstream Applications

Prior to any downstream analysis, you will need to quantitate the amount of protein in your sample. For this we recommend the Quick Start™ Bradford protein assay (Catalog #500-0201). If your downstream analysis technique is negatively impacted by low pH or salts, we recommend that you clean up your sample using Bio-Rad's ReadyPrep™ 2-D cleanup kit (catalog #163-2130).

2-D gel electrophoresis users: The reconstituted elution reagent used to elute your sample is acidic. If using DIGE, adjust pH of eluent to approximately 8.5 with 4 M sodium carbonate. Add approximately 30 µl of 4 M sodium carbonate to 300 µl eluent to bring pH up to 8.5. It is recommended that you then remove excess salts using Bio-Rad's ReadyPrep 2-D cleanup kit (catalog #163-2130) prior to loading sample on an IPG strip. Alternatively, proteins may be eluted by replacing the elution reagent with lysis buffer (25 mM Tris, 4% CHAPS (w/v), 8 M urea, 2 M Thiourea). However, this may result in a decreased number of protein spots.

Loading samples on IPG strips: The total amount of protein to load per strip will vary depending on the sample, the pH range, length of the IPG strip, and the detection system used. In some cases, overloading of protein is acceptable to reveal less abundant proteins of interest. Below is a guideline for protein loads that generally gives acceptable 2-D patterns. Use lower amounts for silver or SYPRO Ruby protein staining and higher amounts for Coomassie Blue staining. In general, the maximum that can be loaded onto an IPG strip is 500 µg for 7 cm, 1 mg for 11 cm, 3 mg for 17 cm/18 cm, and 4 mg for 24 cm.

Recommended Range of Protein Loads for IPG Strips

IPG strip length	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume per strip	125 µl	200 µl	300 µl	315 µl	450 µl
Protein load					
Silver stain	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Protein load					
Coomassie Blue G-250	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg

For SELDI analysis: The sequential elution buffers allow for direct use of samples for SELDI analysis. Use a 1:10 dilution of the extracted sample in the appropriate ProteinChip® binding buffer and incubate the sample for 1 hr with shaking. For on-spot assays, use 0.5 µl of extract and 4.5 µl of the appropriate binding buffer. For Bioprocessor assays, use 5.0 µl of extract and 45.0 µl of appropriate chip binding buffer.

Section 12

References

Boschetti E et al., Reduction of dynamic protein concentration range of biological extracts for the discovery of low-abundance proteins by means of hexapeptide ligand library, *Nature Protocols* 3 (5), 883–890 (2008).

Boschetti E et al., Reduction of the concentration difference of proteins in biological liquids using a library of combinatorial ligands, *Electrophoresis* 26, 3561–3571 (2005).

Boschetti E et al., Reducing protein concentration range of biological samples using solid-phase ligand libraries, *Journal of Chromatography B* 833, 33–40 (2006).

Section 13

Product Information

ProteoMiner Protein Enrichment Kits

Catalog#	Description
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
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
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
163-3009	ProteoMiner Protein Enrichment Introductory Large-Capacity Kit , for processing 50 mg of sample, 2 preps, includes 2 spin columns, wash buffer, elution reagents, collection tubes

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
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

ProteoMiner Kit Accessories

Catalog#	Description
163-3003	ProteoMiner Sequential Elution Reagents , 10 preps, includes reagents only (columns not included), to be used with 163-3006 or 163-3007
163-3012	ProteoMiner Dry Bulk Beads , 0.525 g
732-6207	Mini Bio-Spin Chromatography Columns , empty, 100

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