ProteoMiner™ Sequential Elution Large-Capacity Kit Protocol

Downstream Application Compatibility
The ProteoMiner sequential elution kit is available for the ProteinChip® surface enhanced laser desorption/ionization (SELDI) system or other downstream protein separation analysis methods. **This kit is NOT compatible with 2-D gel electrophoresis.**

Sample Preparation
This kit is optimized for plasma and serum samples; best results are obtained with ≥50 mg protein load. Samples should be free of precipitate. If needed, centrifuge the samples at 10,000 x g for 10 min to clarify.

Perform the following steps to complete the protocol:

Procedure	Add*	Rotate/ Vortex	Centrifuge** 1,000 x g	Repeat Step
Remove top and bottom caps. Centrifuge column for 30–60 sec to remove storage material. Discard flowthrough.	—	—	30–60 sec	—
Add 600 µl wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough and repeat entire step.	600 µl wash buffer	5 min	30–60 sec	x1
Add 1 ml sample to column. Rotate for 2 hr at room temperature. Centrifuge for 30–60 sec. Discard flowthrough and repeat centrifugation.	1 ml sample	2 hr	30–60 sec	—
Add 600 µl wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough and repeat entire step three times.	600 µl wash buffer	5 min	30–60 sec	x3
Add 600 µl DI water on all sides of the column. Centrifuge for 30–60 sec. Discard flowthrough.	600 µl DI water	—	30–60 sec	—
Add 100 µl elution reagent 1 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	100 µl elution reagent 1	10 min	30–60 sec	x2
Add 100 µl elution reagent 2 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	100 µl elution reagent 2	10 min	30–60 sec	x2
Add 100 µl elution reagent 3 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	100 µl elution reagent 3	10 min	30–60 sec	x2
Add 100 µl elution reagent 4 to column. Vortex gently for 5 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	100 µl elution reagent 4	5 min	30–60 sec	x2

* Remove top cap and assure bottom cap is secure on column prior to each addition step. Replace top cap prior to rotating (inverting).
** Remove top and bottom caps before centrifugation. Perform centrifugation steps at 1,000 x g. Refer to manual for detailed protocol.
*** Eluates may be pooled or collected individually; if collected individually, additional collection tubes will be required.

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Sample Preparation

This kit is optimized for plasma and serum samples; best results are obtained with ≥10 mg protein load. Samples should be free of precipitate. If needed, centrifuge the samples at 10,000 x g for 10 min to clarify.

Perform the following steps to complete the protocol:

Procedure	Add*	Rotate/ Vortex	Centrifuge** 1,000 x g	Repeat Step
Remove top and bottom caps. Centrifuge column for 30–60 sec to remove storage material. Discard flowthrough.	—	—	30–60 sec	—
Add 200 µl wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough and repeat entire step.	200 µl wash buffer	5 min	30–60 sec	x1
Add 200 µl sample to column. Rotate for 2 hr at room temperature. Centrifuge for 30–60 sec. Discard flowthrough.	200 µl sample	2 hr	30–60 sec	—
Add 200 µl wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough. **Repeat entire step two times**	200 µl wash buffer	5 min	30–60 sec	x2
Add 200 µl DI water on all sides of the column. Centrifuge for 30–60 sec. Discard flowthrough.	200 µl wash buffer	—	30–60 sec	—
Add 20 µl elution reagent 1 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	20 µl elution reagent 1	10 min	30–60 sec	x2
Add 20 µl elution reagent 2 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	20 µl elution reagent 2	10 min	30–60 sec	x2
Add 20 µl elution reagent 3 to column. Vortex gently for 10 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	20 µl elution reagent 3	10 min	30–60 sec	x2
Add 20 µl elution reagent 4 to column. Vortex gently for 5 min. Place into a clean tube and centrifuge for 30–60 sec. **Repeat entire step twice.****	20 µl elution reagent 4	5 min	30–60 sec	x2

* Remove top cap and assure bottom cap is secure on column prior to each addition step. Replace top cap prior to rotating (inverting).
** Remove top and bottom caps before centrifugation. Perform centrifugation steps at 1,000 x g. Refer to manual for detailed protocol.
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