

ProteoMiner™

Protein Enrichment Small-Capacity Kit Protocol



Reagent Preparation

Prepare elution reagent by adding 610 μ l rehydration reagent to one vial of lyophilized elution reagent. Each vial will contain enough elution reagent for processing two samples.

Sample Preparation

This kit is optimized for plasma and serum samples; best results are obtained with protein concentrations >50 mg/ml. Samples should be free of precipitate. If needed, centrifuge the samples at $10,000 \times g$ 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 μ l wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough. Repeat entire step.	200 μ l wash buffer	5 min	30–60 sec	x1
Add 200 μ l sample to column. Rotate for 2 hr at room temperature. Centrifuge for 30–60 sec. Discard flowthrough.	200 μ l sample	2 hr	30–60 sec	—
Add 200 μ l wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough. Repeat entire step twice.	200 μ l wash buffer	5 min	30–60 sec	x2
Add 200 μ l DI water to column. Rotate column for 1 min. Centrifuge for 30–60 sec. Discard flowthrough.	200 μ l DI water	1 min	30–60 sec	—
Add 20 μ l rehydrated elution reagent to column. Vortex several times over 15 min. Place column into a clean collection tube and centrifuge for 30–60 sec. Repeat entire step twice. ***	20 μ l rehydrated elution reagent	15 min	30–60 sec	x2

* 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 $1,000 \times g$. 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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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 μ l wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough.	600 μ l wash buffer	5 min	30–60 sec	—
Add 600 μ l wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec.	600 μ l wash buffer	5 min	30–60 sec	—
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 (x2)	—
Add 600 μ l wash buffer to column. Rotate column for 5 min. Centrifuge for 30–60 sec. Discard flowthrough. Repeat entire step three times.	600 μ l wash buffer	5 min	30–60 sec	x3
Add 600 μ l DI water to column. Rotate column for 1 min. Centrifuge for 30–60 sec. Discard flowthrough.	600 μ l DI water	1 min	30–60 sec	—
Add 100 μ l rehydrated elution reagent to column. Vortex several times over 15 min. Place column into a clean collection tube and centrifuge for 30–60 sec. Repeat entire step twice.***	100 μ l rehydrated elution reagent	15 min	30–60 sec	x2

* Assure bottom cap is secure on column prior to each addition step. Replace top cap prior to rotating (inverting).

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