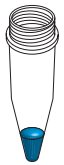
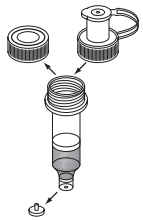
1 Dilute and filter sample



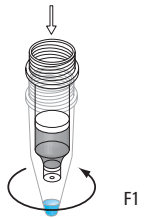
Dilute 7–10-µL human serum sample to 200 µL with Buffer A. Consult cartridge certificate for true sample capacity. Filter through 0.22-µm spin filter.

2 Prepare spin cartridge



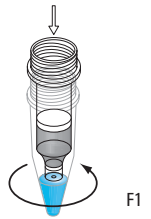
Remove cartridge cap and plug, attach Luer-Lock adapter to cartridge, draw 4 mL of Buffer A into syringe and dispense through cartridge via Luer-Lock, remove excess Buffer A from top of resin bed with transfer pipette.

3 Apply sample



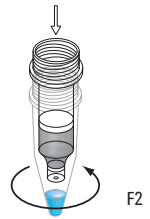
Remove Luer-Lock adapter and add 200-µL diluted serum sample. Cap cartridge loosely or leave open. Place in 1.5-mL collection tube labeled "Flow-through fraction 1" (F1). Centrifuge 1.5 min at 100 × g.

4 Wash and collect flow-through F1



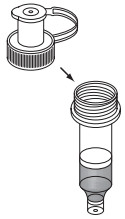
Add 400-µL Buffer A. Centrifuge 2.5 min at 100 × g. Collect in F1 tube.

5 Wash and collect flow-through F2



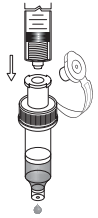
Place spin cartridge in new collection tube labeled "Flow-through fraction 2" (F2). Add 400-µL Buffer A. Centrifuge 2.5 min at 100 × g. Collect in F2 tube.

6 Prepare for elution



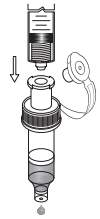
Remove spin cartridge from F2 tube and attach Luer-Lock adapter tightly to top of cartridge.

7 Elute bound fraction



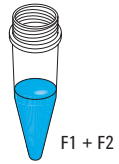
Fill 5-mL Luer-Lock plastic syringe with 2-mL Buffer B and attach to Luer-Lock adapter. Slowly push Buffer B through cartridge to elute bound proteins into new collection tube. Save eluant with targeted high-abundant proteins for analysis or discard.

8 Re-equilibration



Fill new 5-mL plastic syringe with 4-mL Buffer A and attach to Luer-Lock adapter. Slowly push Buffer A through cartridge to re-equilibrate the cartridge for the next sample or store wetted with Buffer A (at 4 °C). Recap both ends for storage.

9 Analyze F1 + F2



Fractions F1 and F2 can be analyzed individually or combined. Concentrate and analyze these fractions containing low-abundant proteins.

For more detailed instructions or information on accessories, refer to the Agilent Multiple Affinity Removal Spin Cartridge Instruction Guide

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Visit www.agilent.com/chem/bioreagents for more products that will help make your proteomics research more efficient:

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Cleanup C18 Pipette Tips

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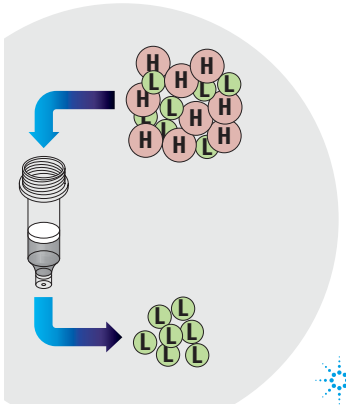
For Human Serum

Agilent Multiple Affinity Removal Spin Cartridge

Part Number 5188-5230

Quick Reference Guide



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