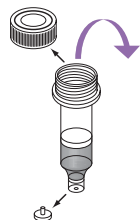


1 Dilute and filter sample



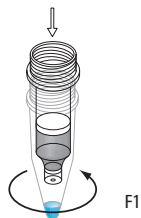
- Dilute 8-10 μL human serum/plasma sample to 200 μL with Buffer A.
- **NOTE:** Consult cartridge certificate for true capacity.
- Filter through 0.22 μm spin filter.

2 Remove buffer



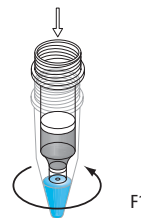
- Remove cartridge cap and plug and remove buffer from top of resin bed with transfer pipette.
- **Never let frits or resin bed run dry.**

3 Apply sample and incubate



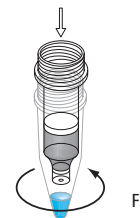
- Add 200 μL diluted serum/plasma sample. Cap cartridge loosely or leave open.
- Place in 1.5 mL collection tube labeled "Flow-through fraction 1" (F1).
- Centrifuge 1 minute at $100 \times g$.
- Remove cartridge from centrifuge and let sit for 5 minutes at room temperature.

4 Wash and collect flow-through F1



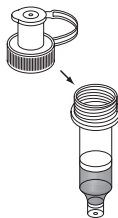
- Add 400 μL Buffer A.
- Centrifuge 2.5 min at $100 \times g$.
- Collect in F1 tube.

5 Wash and collect flow-through F2



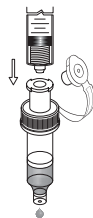
- Place spin cartridge in new collection tube labeled "Flow-through fraction 2" (F2).
- Add 400 μL Buffer A. Centrifuge 2.5 min at $100 \times g$.
- Collect in F2 tube.

6 Prepare for elution



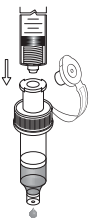
- Remove spin cartridge from F2 tube and attach Luer-Lock adapter tightly to top of cartridge.

7 Elute bound fraction



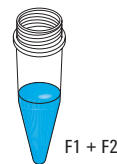
- Fill 5 mL Luer-Lock plastic syringe with 2.5 mL Buffer B and attach to Luer-Lock adapter.
- Slowly push Buffer B through cartridge to elute bound proteins into new collection tube.
- Save eluate with targeted high-abundance proteins for analysis or discard.

8 Re-equilibrate



- Fill new 5 mL plastic syringe with 5 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 $^{\circ}\text{C}$).
- Re-cap 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-abundance proteins.



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For more detailed instructions or information on accessories refer to the Agilent Human 14 Multiple Affinity Removal Spin Cartridge Instruction Guide

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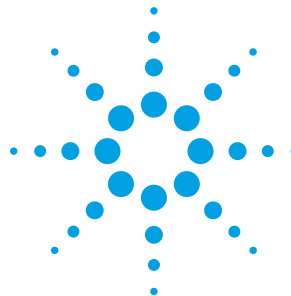
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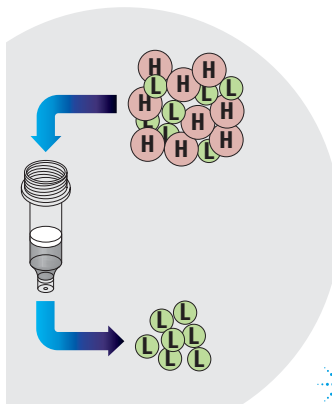
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Part Number 5188-6560

Quick Reference Guide



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