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 buffer or resin bed run dry.

3. Apply sample and incubate
   - Add 200 µL diluted serum/plasma sample. Cap cartridge loosely or let sit for 5 minutes at room temperature.
   - Place in 1.5 mL collection tube labeled “Flow-through fraction 1” (F1).

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 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 °C).
   - Re-cap both ends for storage.

8. Re-equilibrate
   - Fractions F1 and F2 can be analyzed individually or combined. Concentrate and analyze these fractions containing low-abundance proteins.

9. Analyze F1 + F2
   - For more detailed instructions or information on accessories refer to the Agilent Human 14 Multiple Affinity Removal Spin Cartridge Instruction Guide.
Visit www.agilent.com/chem/bioreagents for more products that will help make your proteomics research more efficient:

- Multiple Affinity Removal LC columns for automated serum/plasma processing
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- C18 Cleanup Pipette Tips and Spin Tubes for purifying proteomics samples
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