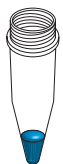
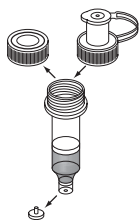


## 1 Dilute and filter sample



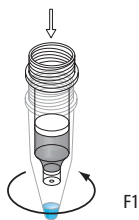
Dilute 25–30- $\mu$ L mouse serum sample to 200  $\mu$ L with Buffer A. Consult cartridge certificate for true sample capacity. Filter through 0.22- $\mu$ m spin filter.

## 2 Prepare spin cartridge



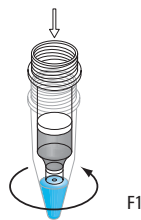
Remove cartridge cap and plug, attach Luer-Lock adapter to cartridge, draw 4 mL of Buffer A into syringe and dispense through cartridge via Luer-Lock, remove excess Buffer A from top of resin bed with transfer pipette.

## 3 Apply sample



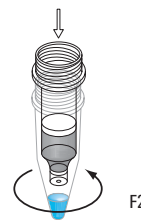
Remove Luer-Lock adapter and add 200- $\mu$ L diluted serum sample. Cap cartridge loosely or leave open. Place in 1.5-mL collection tube labeled "Flow-through fraction 1" (F1). Centrifuge 1.5 min at 100  $\times$  g.

## 4 Wash and collect flow-through F1



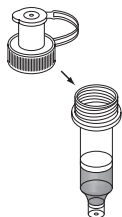
Add 400- $\mu$ L Buffer A. Centrifuge 2.5 min at 100  $\times$  g. Collect in F1 tube.

## 5 Wash and collect flow-through F2



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

## 6 Prepare for elution



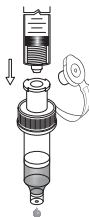
Remove spin cartridge from F2 tube and attach Luer-Lock adapter tightly to top of cartridge.

## 7 Elute bound fraction



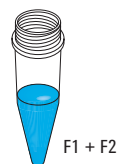
Fill 5-mL Luer-Lock plastic syringe with 2-mL Buffer B and attach to Luer-Lock adapter. Slowly push Buffer B through cartridge to elute bound proteins into new collection tube. Save eluant with targeted high-abundant proteins for analysis or discard.

## 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  $^{\circ}$ 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.



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

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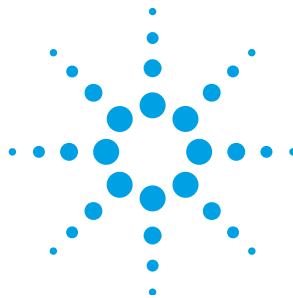
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For Mouse Serum



## Agilent Multiple Affinity Removal Spin Cartridge

Part Number 5188-5289

### Quick Reference Guide

