Supplementary information 1.

1. Depletion workflow. The methods were based on the protocols provided by the company (see http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Bulletin/sep130bul.Par.0001.File.tmp/sep130bul.pdf , access May, 2012).

**Prepare spin column**

Centrifuge 30 sec to remove storage buffer

Column equilibration

(500 μl of dilution buffer, mix the beads and the buffer by inversion and shaking the column, place it on an end-to-end rotator, and incubate at room temperature (RT) for 15 mins)

Centrifuge 30 sec to dry the beads

Sample preparation

(Dilute 20 μl rat plasma with 480 μl of dilution buffer)

Remove particulates with a 0.45 mm spin filter, centrifuge for 1 minute at 10000 *g*.

Add sample to column

(Add 500 μl of diluted sample to the beads, mix the beads and the sample by inversion and shaking the column, place it on an end-to-end rotator, and incubate at RT for 15 mins.)

Centrifuge 30 sec to collect the flow-through as eluted protein 1(1)

Wash the column

（Add 500 μl of dilution buffer to the beads, mix the beads and the buffer by inversion and shaking the column, place it on an end-to-end rotator, and incubate at RT for 15 mins.）

Centrifuge 30 sec to collect the flow-through as eluted protein 1(2)

To remove the non-specifically bound protein

Wash beads with 500 μl of dilution buffer, a total of 3 times. Centrifuge 30 sec to collect the flow-through as 2(1), 2(2),2(3)

Bound protein elution

Repeat 4 times, each time: add 500 μl of stripping buffer to the beads, mix the beads and the buffer by inversion and shaking the column, place it on an end-to-end rotator, and incubate at RT for 5 mins. Centrifuge 30 sec to collect the flow-through as bound protein 3(1), 3(2),3(3),3(4). Pool four eluted samples (~2 ml) and neutralize with 200 ml of 10×neutralization buffer

Regeneration of column resin

Add 600 μl of neutralization buffer, incubate at RT for 6 mins. Centrifuge 30 sec.

Resuspend beads used

Add 0.5 ml of dilution buffer (for storage, buffer contain 0.02% sodium azide)

2. Set up a method to deplete high abundant plasma protein with Seppro rat spin columns.

1. Place the column in a 2 ml collection tube (labelled 25A CT1)
2. Centrifuge the column for 30 seconds at 2,000 rpm, 20°C, with the parameter of accelerate: 9, and brake: 9, with the balance tube, 74 mm fixed angle rotor. Every collection tube was put it on the ice after centrifugation.
3. Place the end cap on the column tip, add 0.5 ml of 1×dilution buffer immediately on the dried beads in the column. Seal the column with the top snap cap. Mix the beads and the sample completely by inversion and shaking the column (shaking 10×3 times, inversion 10 times, shaking 10 times, invert 10 times, shaking 10 times), place it on an end-to-end rotator, speed 14, and incubate at room temperature for 15 minutes. Run one blank samples (buffer only) to remove any residual non-covalently bound IgY from the beads.
4. During the 15 minutes incubation, rat plasma tube was taken out of the -80°C freezer and thawed at room temperature, two aliquots of 20 μl were removed into two 0.5ml tubes (25A DS & 25B DS) separately and the tubes stayed in ice. 480 μl of 1×dilution buffer was added into 25A DS, votexed well, then transferred all the diluted sample into a 0.45μm spin filter (labeled 25A), do not remove the tip. Use another 20 μl pipette to add 15 μl 1×dilution buffer into the 25A DS tube, use 200 μl pipette with the same tip to transfer this 15 μl buffer into filter. The filter was centrifuged with the balance tube for 1 minute at 10,000×g .
5. After 15 minutes incubation of the SEP130 column, inverte the column to remove the end cap, place the column in a 2 ml collection tube (labeled 25A CT2), and centrifuge for 30 seconds at 2,000 rpm to obtain dried beads.
6. Place the end cap on the column tip and adde 0.5 ml of diluted sample immediately on the dried beads in the column. Seal the column with the top snap cap. Mix the beads and the sample completely by inversion and shaking the column (shaking 10×3 times, invert 10 times, shaking 10 times, invert 10 times, shaking 10 times), place it on an end-to-end rotator, and incubate at room temperature for 15 minutes.
7. Invert the column. Remove the end cap, placed the column in a 2 ml collection tube (labeled 25A 1(1)), and centrifuged for 30 seconds at 2,000 rpm.
8. Add 0.5 ml of 1×dilution buffer to the beads. Mixed beads and buffer completely by inversion and shaking the column (shaking 10×3 times, invert 10 times, shaking 10 times, invert 10 times, shaking 10 times). Place it on an end-to-end rotator, and incubate at room temperature for 15 minutes, place the column in a new 2 ml collection tube (labelled 25A 1(2)), centrifuge for 30 seconds at 2,000 rpm. This will obtain maximum yield of the flow-through sample.
9. Inserte the end cap, then add 0.5 ml of 1×dilution buffer, and seal the column with top snap cap. Mix the beads and buffer completely by inversion and shaking the column (shaking 10×3 times, inversion 10 times, shaking 10 times, inversion 10 times, shaking 10 times), place it on an end-to-end rotator, and incubate at room temperature for 5 minutes, remove the end cap while inverting the column, and place it in a 2 ml collection tube (labelled 25A 2(1)). Centrifuge for 30 seconds at 2,000 rpm.
10. Insert the end cap, then add 0.5 ml of 1×dilution buffer, and seal the column with top snap cap. Mix the beads and buffer completely by inversion and shaking the column (shaking 10×3 times, inversion 10 times , shaking 10 times, inversion 10 times, shaking 10 times), remove the end cap while inverting the column, and place it in a 2 ml collection tube (labelled 25A 2(2)). Centrifuge for 30 seconds at 2,000 rpm.
11. Insert the end cap, then add 0.5 ml of 1×dilution buffer, and seal the column with top snap cap. Mix the beads and buffer completely by inversion and shaking the column (shaking 10 × 3 times, inversion 10 times , shaking 10 times, inversion 10 times, shaking 10 times), remove the end cap while inverting the column, and place it in a 2 ml collection tube (labelled 25A 2(3)). Centrifugefor 30 seconds at 2,000 rpm (Step11 and 12 are to remove non-specifically bound protein from beads).
12. Insert the end cap, then add 0.5 ml of 1×stripping buffer, and seal the column with top snap cap. Mix the beads and buffer completely by inversion and shaking the column (shaking 10×3 times, inversion 10 times, shaking 10 times, inversion 10 times, shaking 10 times), place it on an end-to-end rotator, and incubate at room temperature for 3 minutes, remove the end cap while inverting the column, and place it in a 2 ml collection tube (labelled 25A3(1)). Centrifuge for 30 seconds at 2,000 rpm and collect the elute into 25A3(1).
13. Repeat step 15 for 3 times and their elution were collected into 25A 3(2), 25A 3(3), and 25A 3(4) separately.
14. In order to regenerate the spin column after stripping bound proteins, immediately add 0.6 ml of 1×neutralisation buffer to neutralize the beads. Beads and buffer were mixed completely by inversion and shaking the column. They were placed on an end-to-end rotator, and incubated at room temperature for 5 minutes.
15. During the 5 minutes of step 14, use a 200 μl micropipette to transfer the bound protein from 25A 3(2), 25A 3(3), 25A 3(4) all into 25 A 3(1), mix and neutralised with 200μl of 1× neutralization buffer.
16. When the 5 minutes incubating of step 14 is finished, placed it in a 2 ml collection tube (labelled 25A CT3). Centrifuge for 30 seconds at 2,000 rpm.
17. Re-suspend used beads in 0.5 ml of 1×dilution buffer by inversion and shaking the column (shaking 10×3 times, inversion 10 times , shaking 10 times, inversion 10 times, shaking 10 times) , place it on an end-to-end rotator, speed 14 and incubate at room temperature for 15 minutes ( same as step 6, get ready for the second depletion) .
18. During the 15 minutes incubation, took 25B DS out of the ice, diluted with 480μl of 1×dilution buffer, other the same as step 7.
19. When the SEP130 Column is finished with the 15 minutes incubation, invert the column. Remove the end cap, place the column in a 2 ml collection tube (labelled 25B CT2), and centrifuge for 30 seconds at 2,000 rpm to obtain dried beads.
20. For the **second** time depletion for the real sample repeat from step 9 to step 19.
21. Re-suspend used beads in a 0.5 ml of 1× storage buffer by inversion and shaking the column (shaking 10 × 3 times, inversion 10 times, shaking 10 times, inversion 10 times, shaking 10 times), place it on an end-to-end rotator, speed 14, and incubate at room temperature for 5 minutes. Store it in the cold room at 6 OC.