ProteinChip® Serum Fractionation Kit

Instruction Manual

Catalog #K10-00007

BIO-RAD

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Life Science Group

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Introduction

The ProteinChip serum fractionation kit is designed to facilitate the analysis of crude serum samples by fractionating proteins based on their biophysical properties.

The kit allows high-throughput fractionation by using anion exchange media in a 96-well microplate format. The anion exchange support is supplied in a 96-well ProteinChip Q filtration plate and requires rehydration before use. The samples are added to the plate and then eluted in a stepwise manner by altering the pH of the wash buffer until six fractions are collected. The fractions can then be analyzed on a ProteinChip array using a profiling protocol. Each of the six fractions is collected twice, and the two collections are pooled. This helps to ensure that the pH changes appropriately, and also results in greater reproducibility in the fractionation.

The fractions can be analyzed in your particular profiling application. If you are using multiple array types or conditions, we recommend that the same fraction is profiled at one time to avoid multiple freeze-thaw cycles.

Materials

Materials Included

- ProteinChip U9 buffer (9 M urea, 2% CHAPS, 50 mM Tris-HCl, pH 9), 20 ml
- Rehydration buffer (50 mM Tris-HCl, pH 9), 250 ml
- Wash buffer 1 (50 mM Tris-HCl, 0.1% OGP, pH 9), 20 ml
- Wash buffer 2 (50 mM HEPES, 0.1% OGP, pH 7), 30 ml
- Wash buffer 3 (100 mM Na acetate, 0.1% OGP, pH 5), 30 ml
- Wash buffer 4 (100 mM Na acetate, 0.1% OGP, pH 4), 30 ml
- Wash buffer 5 (50 mM Na citrate, 0.1% OGP, pH 3), 30 ml
- Wash buffer 6 (33.3% isopropanol, 16.7% acetonitrile, 0.1% trifluoroacetic acid), 30 ml
- ProteinChip Q filtration plate, filled with dehydrated anion exchange Q ceramic HyperD F sorbent, 1

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- Microplate sealing strips, 10
- Instruction manual

Note: The volume of supplied reagents allow for processing of the 96-well filtration plate in a maximum of two runs (2 x 48 samples). ProteinChip serum fractionation kit replacement buffers (catalog #K10-00008) are available to order.

Materials Needed but Not Included

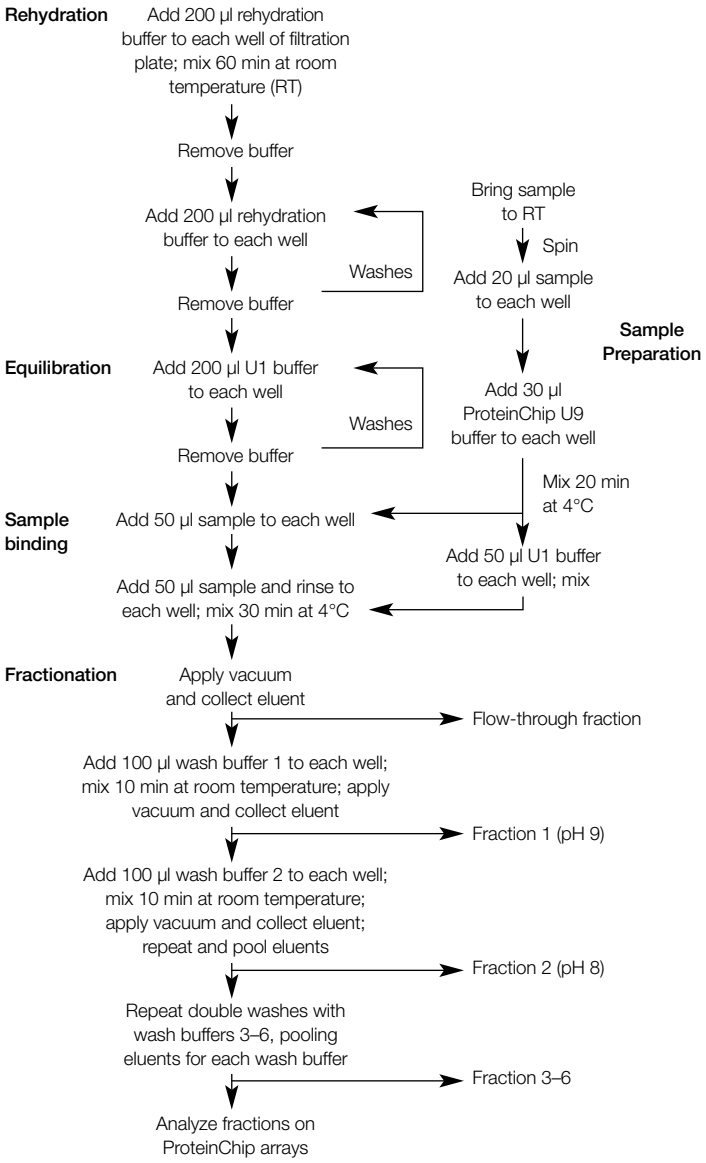
- V-bottom 96-well microplate labeled samples
- V-bottom 96-well microplates labeled F1–F6
- 96-well microplate for collection of waste
- Adhesive sealing film for microplates
- 12-column partitioned buffer reservoir
- Pipet tips
- MicroMix 5 plate and tube shaker (Diagnostic Products Corp.)
- Biomek 3000 workstation integration package (catalog #Z33-00030)
- Vacuum manifold (for manual use)

Storage

Table 1. Storage conditions for kit components.

Item	Storage
ProteinChip U9 buffer	–20 to –50°C
Rehydration and wash buffers	2–8°C
ProteinChip Q filtration plate	2–8°C

Protocol Flow Chart



Detailed Use Protocol

The following protocol can be used with the Biomek 2000 or 3000 laboratory automation workstation or can be performed manually using a vacuum manifold. We recommend a vacuum setting of 15 in Hg.

Notes:

1. When using the filtration plate in the MicroMix 5, make sure that the plate is securely held in the manifold and that the bottom of the plate is not touching the surface of the mixer.
2. The settings recommended for the MicroMix 5 may vary from mixer to mixer. If you experience problems with mixing, you may need to adjust the settings. See Appendix B for instructions.
3. When removing the foil seal from the ProteinChip Q filtration plate, you may notice some of the sorbent adhered to the foil seal. The amount should be relatively uniform across all wells. This is normal and has not been found to adversely affect the performance of the kit.

Step 1: Q Ceramic HyperD F Sorbent Rehydration

The filtration plate should be used directly after rehydration.

1. Tap the filtration plate on the workbench several times to make sure that all of the dry Q ceramic HyperD F sorbent is settled to the bottom of the plate.
2. Take the filtration plate out of the pouch and carefully remove the top seal on the filtration plate.

Note: If using only part of the filtration plate, with a sharp blade remove only enough of the foil seal to reveal the columns needed. Alternatively, remove the whole seal, and reseal the unneeded wells with the microplate strips provided. While processing samples, only the wells in use should be uncovered. After using part of the plate, reseal the used wells with microplate strips and mark those columns as used.

3. With an 8-channel pipet, add 200 µl of rehydration buffer to each well.
4. Carefully seal the plates with microplate sealing strips and shake by hand for a few minutes (some liquid may come through the membrane) until the sorbent appears to be resuspended in solution.
5. Mix the filtration plate on the MicroMix 5 (form 48, amp 7) for 60 minutes at RT. Carefully remove the sealing strips. Place plate on vacuum collar and then place the vacuum collar and plate on vacuum manifold.

Note: It is extremely important to mix the sorbent and rehydration buffer well to avoid plugging wells during fractionation. Check visually that mixing is adequate. If necessary, adjust the MicroMix form and amp settings. Mixing should be vigorous enough to ensure that all sorbent is in contact with buffer without the mixture reaching the top of the well. If mixing is still not adequate after changing the MicroMix settings, it may be necessary to adjust settings in the MicroMix 5 software (see Appendix B).

Step 2: Sample Preparation

1. Bring serum samples to ambient temperature. Spin at 20,000 g for 10 minutes at 2–8°C.
2. Aliquot 20 µl of serum sample to each well of a standard V-bottom 96-well microplate.
3. Add 30 µl of ProteinChip U9 buffer to each well.
4. Cover microplate with adhesive sealing film and mix on the MicroMix 5 (set at 20, 5, 20) for 20 minutes at 2–8°C.

Step 3: Preparation of U1 Buffer

1. Add 10 ml of ProteinChip U9 buffer solution to 80 ml of rehydration buffer (50 mM Tris-HCl) to produce U1 buffer (1 M urea, 0.2% CHAPS, 50 mM Tris-HCl, pH9). The volume is enough for 1 complete plate. If you are using part of the plate, adjust volume accordingly.

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Step 4: Equilibration of Q Ceramic HyperD F Sorbent With U1 Buffer

1. After step 1.4, place the waste collection plate underneath the filtration plate and apply a vacuum to remove the buffer from the filtration plate.
2. Add 200 µl of rehydration buffer to each well.
3. Apply vacuum to remove the buffer in the filtration plate.
4. Repeat steps 4.2 and 4.3 three times with rehydration buffer and an additional three times with U1 buffer. Empty the waste plate under the filtration plate as necessary between washes.
5. The sorbent in the filtration plate is now ready to bind sample.

Step 5: Binding Sample With Sorbent

1. Pipet 50 µl of sample from each well of the sample microplate to the corresponding well in the 96-well filtration plate.
2. Add 50 µl of U1 buffer to each well of the sample microplate.
3. Mix 5 times.
4. Pipet 50 µl from each well of the sample microplate to the corresponding well in the 96-well filtration plate.

Note: This step is included because there is a dead volume when pipetting with the robot. When the robot pipets to collect the sample initially, it does not collect all of the material. The addition of 50 µl of U1 buffer and mixing allows the residual material to be collected and added to the first 50 µl.

5. Cover the filtration plate with adhesive sealing film and mix on the MicroMix 5 (set at 20, 7, 30) for 30 minutes at 2–8°C.

Step 6: Fraction Collection

Note: To avoid cross-contamination between wells, apply adhesive sealing film on the microplate during the mixing step. Remove the film before applying a vacuum, and replace with a new piece for each mixing.

Fraction 1

1. Place the 96-well microplate labeled F1 underneath the filtration plate.
2. Apply the vacuum and collect the flowthrough into the F1 plate.
3. Add 100 µl of wash buffer 1 to each well of the filtration plate.
4. Mix for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
5. Apply the vacuum and collect the eluent into the F1 plate.

Note: Fraction 1 contains the flowthrough and the pH 9 eluent.

Fraction 2

1. Add 100 µl of wash buffer 2 to each well of the filtration plate.
2. Mix for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
3. Place the 96-well microplate labeled F2 underneath the filtration plate.
4. Apply the vacuum and collect the eluent into the F2 plate.
5. Repeat procedures 1–4.

Note: Fraction 2 contains the pH 7 eluent.

Fraction 3

1. Add 100 µl of wash buffer 3 to each well of the filtration plate.
2. Mix for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
3. Place the 96-well microplate labeled F3 underneath the filtration plate.
4. Apply the vacuum and collect the eluent into the F3 plate.
5. Repeat procedures 1–4.

Note: Fraction 3 contains the pH 5 eluent.

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Fraction 4

1. Add 100 µl of wash buffer 4 to each well of the filtration plate.
2. Mix for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
3. Place the 96-well microplate labeled F4 underneath the filtration plate.
4. Apply the vacuum and collect the eluent into the F4 plate.
5. Repeat procedures 1–4.

Note: Fraction 4 contains the pH 4 eluent.

Fraction 5

1. Add 100 µl of wash buffer 5 to each well of the filtration plate.
2. Mix for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
3. Place the 96-well microplate labeled F5 underneath the filtration plate.
4. Apply the vacuum and collect the eluent into the F5 plate.
5 Repeat procedures 1–4.

Note: Fraction 5 contains the pH 3 eluent.

Fraction 6

1. Aliquot wash buffer 6 into the buffer tray.
2. Mix 100 µl of wash buffer 6 to each well of the filtration plate.
3. Vortex for 10 minutes on the MicroMix 5 (set at 20, 7, 10) at RT.
4. Place the 96-well microplate labeled F6 underneath the filtration plate.
5. Apply the vacuum and collect the eluent into the F6 plate.
6. Repeat procedures 2–5.

Note: Fraction 6 contains the organic solvent eluent.

Step 7

Seal the six collection microplates and store until proceeding with the ProteinChip array binding protocol. If the samples are to be analyzed within 24 hours, store at 4°; longer term storage should be at –20°C.

Step 8

Dispose of the sample plate as biohazard waste if human serum sample is used.

Step 9

If you only used part of the filtration plate, put the filtration plate back in the pouch; seal and store at 4°C.

Ordering Information

Catalog #	Description
K10-00007	**ProteinChip Serum Fractionation Kit**, includes 1 x 96-well ProteinChip Q filtration plate packed with Q ceramic HyperD F sorbent, buffers, sealing strips, instructions
K10-00010	**ProteinChip U9 Buffer**, for serum fractionation kit, 20 ml
K10-00008	**ProteinChip Serum Fractionation Kit Replacement Buffers**, includes wash buffers 1–6
C54-00018	**ProteinChip Q Filtration Plate**, 1 x 96-well
C57-30075	**ProteinChip CM10 Arrays**, A-H format, 12
Z33-00030	**Biomek 3000 Workstation Integration Package**, includes Biomek 3000 workstation Windows XP operating system and 17" flat-panel monitor, integrated custom mixer/shaker, Biomek software with CFR 21 Part 11 compliance capability, wash station, 8-channel pipet, wash tools, vacuum system

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Appendix A: Performance Specification

When the ProteinChip serum fractionation kit is used according to this protocol, each sample processed will produce six fractions (see Figure 1).

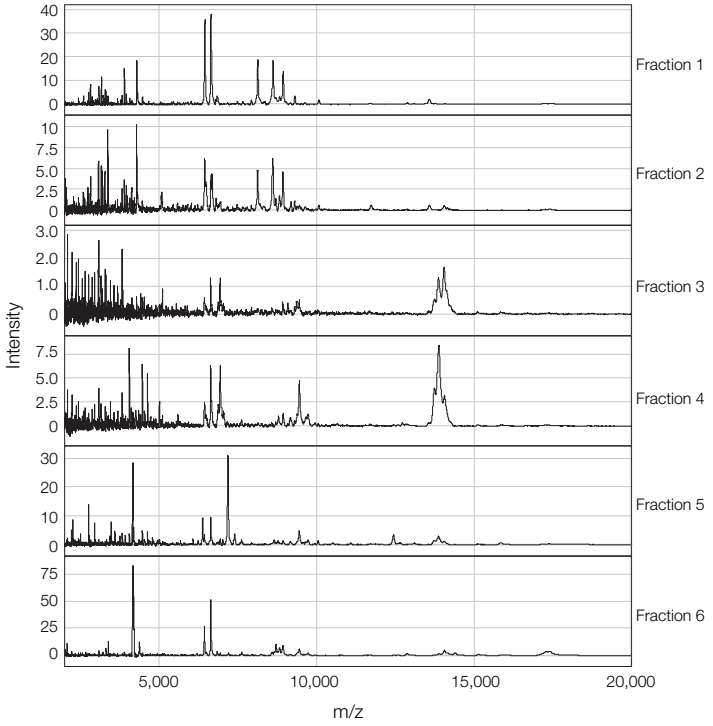

Fig. 1. Example fractionation of human serum sample. Profiled on ProteinChip CM10 arrays (2–20 kD).

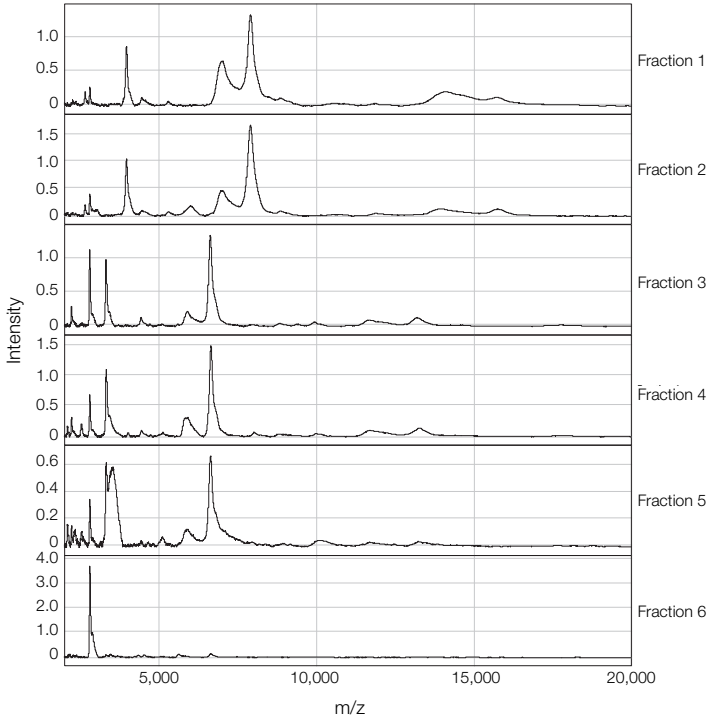

Fig. 2. Example fractionation of human serum sample. Profiled on ProteinChip CM10 arrays (20–200 kD).

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Appendix B: Adjusting MicroMix 5 Settings

In some instances when rehydration of the sorbent in the plate has been problematic, the settings of the MicroMix shaker can be adjusted to improve the vigorousness of the shaking. Adjustments are made to the Gain Factor in the advanced hardware settings of the MicroMix 5 software. Generally, the manufacturer of the shaker recommends that advanced hardware settings not be changed. However, some advanced users may find changing these settings useful during troubleshooting.

CAUTION: Changing the Gain Factor and/or Damping Factor affects the calibration of the shaker, and recalibration is necessary.

To change the Advanced Hardware settings:

1. Choose **Settings>Advanced Hardware**. Warning appears.

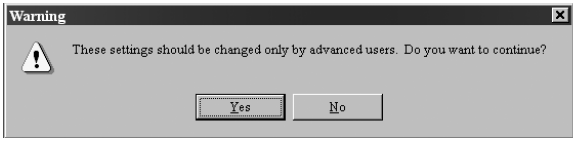

Fig. 3. Advanced Hardware settings warning dialog box.

2. Choose **Yes**. The Advanced Hardware dialog box appears.

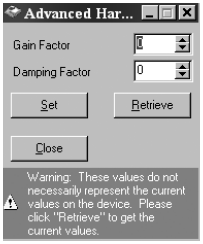

Fig. 4. Advanced Hardware dialog box.

3. Enter the Gain Factor.

   The Gain Factor is the scaling of the amplitude of shake. It is adjusted by changing the multiplication factor used by the firmware for amplitude control. The nominal value of the Gain Factor is factory set and should normally be 14. The Gain Factor should not go below 10 or above 18.

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