# ProteomeLab™ IgY-R7 Proteome Partitioning Kits

Standard Operating Protocol

**A25403AB**

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Beckman Coulter, Inc.
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## Licenses and Trademarks

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# ProteomeLab™ IgY-R7 Proteome Partitioning Kits

## Introduction

The ProteomeLab IgY-R7 Proteome Partitioning kits are based on affinity columns using avian antibody (IgY)-antigen interactions and optimized buffers for sample loading, washing, eluting and regenerating. They are specifically designed to remove seven highly abundant proteins from rodent biological fluids such as serum, plasma, and cerebral spinal fluid (CSF).

This technology enables removal of albumin, IgG, α1-antitrypsin, IgM, transferrin, haptoglobin and fibrinogen in a single step. The targeted highly abundant proteins are simultaneously removed by the immobilized specific IgY antibodies when complex biological samples are passed through the column. Selective immunoaffinity partitioning provides an enriched pool of lower abundant proteins for downstream proteomics analysis. Specific removal of seven highly abundant proteins partitions up to 80% of total protein mass from mouse or rat serum or plasma. The lower abundant proteins in the flow-through fractions and the highly abundant proteins in the bound/eluted fractions can be collected and further fractionated with the ProteomeLab PF 2D System or other fractionation or analytical methods. The technology uses physiological buffers for binding and washing, and avoids urea and other chaotropic agents for elution that can precipitate at low temperature. The enriched proteome, which includes medium and low abundance proteins, is the primary target for discovery and validation of biomarkers, toxicoproteomics, animal disease models, drug targets, and therapeutic proteins and antibodies.

# ProteomeLab IgY-R7 Requirements and Yields

The ProteomeLab IgY-R7 SC, IgY-R7 LC2, and IgY-R7 LC10 Proteome Partitioning kits are selected based on the following:

- the volume of rat or mouse biological fluids needed to yield the target quantity of partitioned protein for subsequent analysis
- the sample throughput requirements
- the availability of liquid chromatography equipment

The IgY-R7 SC spin column kit is based on centrifugation and has a capacity of 10 µL mouse/15 µL rat serum or plasma per spin column cycle. The expected yield of a sample partitioned of the 7 highly abundant proteins is about 150 - 250 µg. We recommend the use of the ProteomeLab™ SP Sample Preparation System with the IgY-R7 SC kit.

The IgY-R7 LC2 column kit requires liquid chromatography equipment and has a capacity of 20 µL mouse/40 µL rat serum or plasma per LC column cycle. The expected yield of a sample partitioned of the 7 highly abundant proteins is about 300 - 600 µg. We recommend the use of the ProteomeLab™ PPS Proteome Partitioning System with the IgY-R7 LC2 column kit.

The IgY-R7 LC10 column kit also requires liquid chromatography equipment and has a capacity of 100 µL mouse/200 µL rat serum or plasma per LC column cycle. The expected yield of a sample partitioned of the 7 highly abundant proteins is about 1.5 - 3 mg. Samples can be pooled for ProteomeLab PF 2D (recommended 1 - 5 mg) or other fractionation methods and can be analyzed by 2D gel electrophoresis or LC-MS methods. We recommend the use of the ProteomeLab™ PPS Proteome Partitioning System with the IgY-R7 LC10 column kit.

Under proper conditions of sample preparation and affinity chromatography, each column is capable of 100 cycles before replacement is needed. There is sufficient buffer supplied for 100 cycles per column.

## Additional Proteome Partitioning Kits

ProteomeLab IgY Proteome Partitioning kits in SC, LC2, and LC10 formats containing IgY antibodies directed against multiple abundant proteins are also available for:

- IgY-12 for primate samples partitioning twelve highly abundant proteins

ProteomeLab IgY Proteome Partitioning Spin Column kits containing IgY antibodies directed against single abundant proteins are also available for:

- Anti-RSA (rat serum albumin)
- Anti-IgG-Fc
- Anti-transferrin
- Anti-fibrinogen
- Anti-HDL
- Anti-BSA (bovine serum albumin)
- Anti-HSA (human serum albumin)

# IgY-R7 Spin Column

**Reorder number: A25626**

**Observed Capacity*: 10 µL mouse serum or plasma (per run)**
**15 µL rat serum or plasma (per run)**

| | |
|---|---|
| IgY-R7 SC<br><br>2 x 1.2 mL IgY-R7 Microbeads packed in a spin column | Spin Column consisting of anti-rat serum albumin, anti-IgG, anti-fibrinogen, anti-transferrin, anti-α1-antitrypsin, anti-haptoglobin, and anti-IgM. Affinity-purified anti-IgY antibodies are covalently conjugated through their Fc portion to 60 µm polymeric microbeads as a 50% resin in Dilution Buffer with 0.02% Sodium Azide. |
| 6 empty spin columns<br>4 spin column end caps | Used for transferring resin after every 25 cycles and for sealing spin column tips. |
| 1x Dilution Buffer (4 x 180 mL) | Tris Buffered Saline: 10 mM Tris-HCl, pH 7.4, 150 mM NaCl. For sample dilution, washing and equilibrating column, and rinsing pipette tips during resin transfer. |
| 1x Stripping Buffer (2 x 120 mL) | 0.1 M Glycine-HCl, pH 2.5 for stripping off bound proteins from column. |
| 10x Neutralization Buffer (2 x 20 mL) | 1 M Tris-HCl, pH 8.0 for neutralizing column and eluted proteins. |
| 1,000 Collection tubes | 2 mL collection tube vials. |

## ProteomeLab Spin Column Common Characteristics

| | |
|---|---|
| Antibody Produced From: | Chicken, polyclonal. Antigen affinity-purified |
| Column Storage Buffer: | Dilution Buffer with 0.02% sodium azide |
| Column Storage Instruction: | 2-8°C<br>**Do not freeze.** |
| Recommended Application: | Plasma or serum protein separation, immunoprecipitation. |

*The IgY Microbeads are reusable for 100 times under proper conditions, including transfer of resin into a fresh spin column after every 25 uses.

**Important: The Neutralization buffer is shipped as 10x buffer. Use undiluted buffer to neutralize eluted proteins. Dilute 10 mL of this buffer to 1x with 90 mL of HPLC grade water prior to use for neutralizing the spin column.**

## Materials Required but Not Supplied in this Kit

- ProteomeLab™ SP Sample Preparation System including one F2402H Fixed Angle rotor
- End-to-end Rotator (e.g., Glas-Col Catalog No. 099A-RD4512)
- Pipette and pipette tips (1-200 µL and 200-1000 µL)
- Column Storage Dilution Buffer with 0.02% (w/v) Sodium Azide
- Microcon YM-3 Centrifugal Filter Device (Millipore Catalog No. 42420 for the 8 pack, Catalog No. 42403 for the 24 pack or Catalog No. 42404 for the 100 pack)
- HPLC grade water
- Gloves and protective eyewear

## Safety Issues

When preparing biological samples using ProteomeLab Columns, follow general guidelines for handling biological materials and wear the appropriate protective attire.

## Methods

### Immunocapture of 7 Abundant Rodent Serum/Plasma Proteins

1. Dilute a 15 µl rat sample in 485 µl Dilution Buffer, or 10 µL mouse sample in 490 µL to get a final volume of 500 µl (1:33 or 1:50 dilution).
2. For the first use, snap off the tip from the column and place the column in a 2 mL centrifuge collection tube.
3. Before every run, ensure all buffer bottles are free of turbidity and precipitation. Replace them if present.
4. Centrifuge the column for 30 sec. at 400 x *g* to obtain dried beads.
5. Place the end cap on the column tip. Immediately add 0.5 mL diluted sample to the dried beads in the column. Seal the column with the top snap cap.
6. Mix the beads and the sample completely by inverting and shaking the column.
7. Place the sample on an end-to-end rotator and incubate it at room temperature for 15 min.
8. Invert the column. Remove the end cap and place the column in a 2 mL centrifuge collection tube. Centrifuge for 30 seconds at 400 x *g*. Collect the flow-through (partitioned) sample for further analysis.
9. To obtain maximum yields of flow-through samples, an optional wash step can be applied. Add 0.5 mL Dilution Buffer to the beads. Mix beads and buffer completely by inverting and shaking the column. Centrifuge for 30 seconds at 400 x *g*. Collect and combine with the flow-through sample from step 8 for further analysis.
10. Concentrate the sample using the Microcon YM-3 centrifugal filter device. Refer to the protein concentration instructions provided by the manufacturer.

## Stripping of Bound Proteins

1. To remove proteins non-specifically bound to microbeads, wash the beads with Dilution Buffer, a total of 3 times. For each wash, always first insert the end cap, and then add 0.5 mL Dilution Buffer and seal the column with top snap cap. Mix the beads and buffer completely by inverting and shaking the column, remove the end cap while inverting the column and place it in a 2 mL ProteomeLab centrifuge collection tube. Centrifuge for 30 seconds at 400 x *g* and save the flow-through for further analysis.
2. Strip off bound proteins from beads using Stripping Buffer a total of 2 times. For each elution, place the end cap to the column first after centrifugation, then add 0.5 mL Stripping Buffer and seal the column with top snap cap. Mix the beads and buffer completely by inverting and shaking the column, incubate at room temperature for 3 min., remove the end cap while holding the column upside down and place it in a 2 mL ProteomeLab centrifuge tube. Centrifuge for 30 seconds at 400 x *g* and collect the eluant.

   **It is crucial for column stability to IMMEDIATELY neutralize the beads.**
   **See "Spin Column Regeneration" on page 6.**

3. Pool two eluted samples (total ~1 mL) and neutralize with 100 µl of 10x Neutralization Buffer. Samples can be further concentrated using the centrifugal filter device to achieve the desired concentration and volume. Refer to the protein concentration instructions provided by the manufacturer.

## Spin Column Regeneration

1. To regenerate ProteomeLab beads after stripping bound proteins as described above, **immediately** neutralize beads with 0.6 mL of 1:10 diluted Neutralization Buffer. Mix beads and buffer completely by inverting and shaking the column. Incubate at room temperature for 5 min.
2. Spin down beads in the column for 30 seconds at 400 x *g*.
3. Resuspend beads in 0.5 mL Dilution Buffer. Beads are ready for storage at 4°C or the next separation for up to 7 days. For storage of regenerated beads for more than 7 days, it is suggested that the Dilution Buffer contain 0.02% (w/v) sodium azide. In either case, seal the column with an end cap prior to storage.
4. Keep careful track of the number of times each spin column is used. After cycles 25, 50, and 75, transfer resuspended resin (1.2 mL) with a disposable 1000 µL pipette tip into a fresh empty spin column.
5. To efficiently transfer beads to a new spin column after each set of 25 runs, we suggest re-suspending the beads with 500 µl Dilution Buffer, then transferring them to a new spin column. Centrifuge the new column in a collection tube for 30 seconds at 400 x *g*, rinse the old column with 500 µl Dilution Buffer, and transfer with the same tip to the new spin column to completely recover residual microbeads.

## Troubleshooting

| Symptom | Possible Cause | Corrective Action |
|---|---|---|
| Reduction in protein binding capacity | Column may have not been regenerated well enough from previous runs. | Elute bound proteins with Stripping Buffer for 2 additional column volumes, and then immediately neutralize and re-equilibrate the column with 1x Neutralization Buffer and Dilution Buffer. |
| | Buffers contaminated with bacteria or fungi. | Carefully check for signs of biological growth and precipitates in the Buffers. Replace with fresh Buffers for optimized column performance. |
| | Diminished resin bed volume due to serial transfer into new spin columns. | After each set of 25 runs, it is suggested to re-suspend the beads with 500 µL Dilution Buffer, then transfer them to a new spin column. Centrifuge the new column in a collection tube for 30 seconds at 400 x *g*, rinse the old column with 500 µL Dilution Buffer and transfer with the same tip to the new spin column to completely recover residual microbeads. |

## Additional Technical Support

### In North America:

Contact Beckman Coulter Technical Support
1.800.742.2345
proteomelab@beckman.com

### Worldwide:

Contact your Beckman Coulter Technical Support Associate.

## Expected Performance

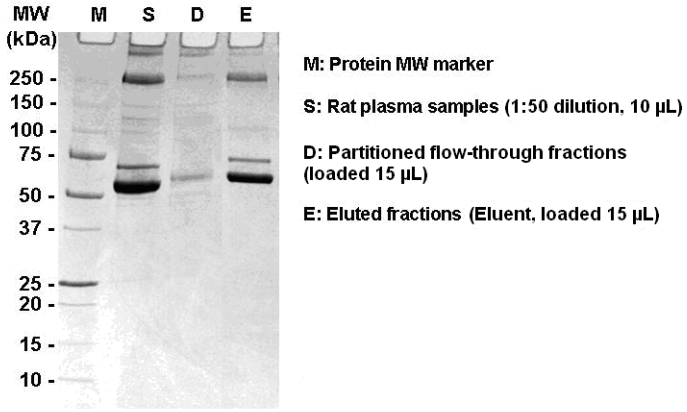


**Figure 1: Affinity Separation of Seven Abundant Proteins from Rat Plasma.** ProteomeLab IgY-R7 spin column specifically removes RSA, IgG, fibrinogen, α1-antitrypsin, transferrin, haptoglobin and IgM from rat plasma or serum. 4-20% SDS PAGE under non-reducing condition with Coomassie Blue staining.

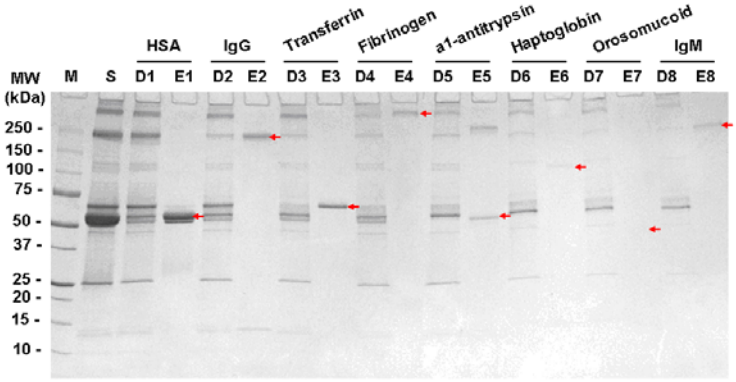


**Figure 2: Serial Partitioning of 8 Mouse Plasma Proteins by Anti-human IgY Microbeads.** Mouse plasma was sequentially treated by 8 individual IgY microbead spin columns against human plasma proteins. IgY microbeads and their order used in the sequential depletion process are as labeled. D1 fraction (flow-through of anti-HSA column) was applied to the next column (anti-IgG) and the flow-through was collected as D2. The same process was repeated from D2 to D8 by applying the flow-through of one column to the next column. Fractions E1-E8 were collected by eluting the bound proteins from the columns. Collected fractions were analyzed by SDS-PAGE under non-reducing conditions and were visualized by Coomassie Blue staining. Ten microliters (10 µl) of unfractionated mouse plasma (1:50 diluted with Dilution Buffer Lane S), 10 µl each flow-through fractions (Lanes D1 to D8), and 20 µl each of eluted fractions (Lanes E1 to E8) were loaded on the gel. M, Molecular weight marker. The arrows indicate the target proteins.

# ProteomeLab IgY-R7 LC2 Proteome Partitioning Kit

## Using these Instructions

Both of the ProteomeLab LC Proteome Partitioning Kits have specific measurements, requirements, and protocols. After noting the Protocol for this kit, See “ProteomeLab LC Partitioning Kit General Documentation” on page 21, for Troubleshooting and Recommendations.

**Reorder number: A25404**

**Observed Capacity*: 20 µL mouse serum or plasma (per run)**
**40 µL rat serum or plasma (per run)**

| Kit Contents | Product Description |
|---|---|
| IgY-R7 LC2<br><br>6.4 × 63 mm, Affinity Column | LC Column consisting of anti-rat serum albumin, anti-IgG, anti-fibrinogen, anti-transferrin, anti-α1-antitrypsin, anti-haptoglobin, and anti-IgM. Affinity-purified anti-IgY antibodies are covalently conjugated through their Fc portion to 60 µm polymeric microbeads as a 50% resin in dilution buffer with 0.02% sodium azide. |
| 10x Dilution Buffer (200 mL) | Tris Buffered Saline: 100 mM Tris-HCl, pH 7.4, 1.5 M NaCl. For sample dilution, washing and equilibrating column. |
| 10x Stripping Buffer (180 mL) | 1 M Glycine-HCl, pH 2.5 for stripping off bound proteins from column. |
| 10x Neutralization Buffer (2 x 80 mL) | 1 M Tris-HCl, pH 8.0 for neutralizing column and eluted proteins. |
| 100 Spin Filters, 0.45 µm | For sample clean up before loading column to remove sample particulates and extend column life. |

*The IgY Microbeads are reusable for 100 times under proper conditions.

**IMPORTANT: All buffers are shipped as 10x buffer. Dilute buffers to 1x with HPLC grade water prior to use. For neutralization of eluted proteins, retain 50 mL of undiluted 10x Neutralization Buffer.**

## ProteomeLab LC Column Common Characteristics

| | |
|---|---|
| Antibody Produced From: | Chicken, polyclonal. Antigen affinity-purified |
| Column Storage Buffer: | Dilution Buffer with 0.02% sodium azide. |
| Column Storage Instruction: | 2-8°C<br>**Do not freeze.** |
| Recommended Application: | Plasma or serum protein separation, immunoprecipitation. |

## Column Specifications

| | |
|---|---|
| Column Name: | IgY-R7 LC2 |
| Column Size: | 6.4 x 63 mm (2 mL bed volume) |
| Column body materials: | Polycarbonate column cylinder, Polyethylene frit, Tefzel caps, Buna-N-rubber O rings, Delrin nut fittings, ETFE ferrules, and Teflon PFA tubing. |
| Column capacity: | 20 µL mouse serum/plasma<br>40 µL rat serum/plasma |
| Maximum pressure: | 100 PSI (6 bar) |
| Operating temperature: | 18-25° C |
| Column packing material: | Antibody-modified resin |
| Immobilized ligands: | Affinity-purified IgY polyclonal antibodies to rat serum albumin, IgG, fibrinogen, transferrin, α1-antitrypsin, haptoglobin, and IgM. |
| Flow rate range: | 0.1-1 mL/min. |

## Materials Required but Not Supplied in this Kit

- ProteomeLab SP Sample Preparation System, including one F2402H Fixed Angle rotor and one SX4250 Swinging Bucket rotor with Adapter PN 392257 for a 15 mL conical tube
- ProteomeLab PPS Proteome Partitioning System
- Pipette and pipette tips (1-200 µL and 200-1000 µL)
- Column Storage Dilution Buffer with 0.02% (w/v) Sodium Azide
- Amicon Ultra-4 Centrifugal Filter Device (Millipore, Catalog No. UFC8 005 08 for the 8 pack, or Catalog No. UFC8 005 24 for the 24 pack)
- HPLC grade water
- 10 mL polypropylene tubes for fraction collection
- 1000 mL graduated cylinder
- Gloves and protective eyewear

## Safety Issues

When preparing biological samples using ProteomeLab Columns, follow general guidelines for handling biological materials and wear the appropriate protective attire.

## Methods

### Protocol for 6.4 x 63 mm LC2 Column (Column capacity: 20 µL mouse or 40 µL rat serum/plasma)

1. Dilute 100 ml of each stock 10x buffer with 900 ml HPLC grade water.
2. Transfer each 1x buffer to a separate 1 liter solvent bottle.
3. Before every run, ensure all buffer bottles are free of turbidity and precipitation. Replace them if these conditions are present.
4. Prime the solvent lines with the three 1x diluted buffers (Dilution, Stripping and Neutralization) according to the ProteomeLab PPS Operators Manual or the instructions of other chromatographic systems.
5. Start the 32 Karat software and select the `IgYLC2.met` method file on the ProteomeLab PPS according to the User's Guide, or program the chromatographic system, using the instructions found in the programming guidelines.
6. Set up the LC timetable. See "Appendix A: IgY-R7 LC2" on page 23 for guidelines on non-ProteomeLab PPS Systems.
7. Attach the column and set the injector to the inject position.

8. Before the first run of the day, after removing the column from the refrigerator, equilibrate with 1x Dilution Buffer for 20 min. at a flow rate of 1.0 mL/min. at room temperature to ensure a flat baseline.
   - The first time the column is used and after temperature equilibration, perform two complete buffer runs without injecting a sample.
9. Dilute the 20 µl mouse sample in 105 µL of 1x Dilution Buffer (1 to 6 dilution), or 40 µL rat sample in 85 µL of 1x Dilution Buffer (1 to 3 dilution) to get a final volume of 125 µL.
10. Remove any sample particulates using a 0.45 µm spin filter. Centrifuge for 1 minute at 9200 x *g*.
11. Inject 125 µL of the diluted sample and start the separation method.
12. Collect flow-through and eluted fractions. Add 1/10 volume 10x Neutralization Buffer (0.35-0.45 mL) to eluted fraction (3.5-4.5 mL). Store both fractions at -70°C if they are not immediately analyzed.
13. If the column is not going to be reused within 7 days, equilibrate the column with 1x Dilution Buffer that contains 0.02% (w/v) sodium azide. Start the 32 Karat software and select `IgYLC2_store.met` method file on the ProteomeLab PPS System, or pump the 1x Dilution Buffer with 0.02% sodium azide at a flow rate of 1.0 mL/min. for 10 min. through the column on other chromatographic systems. Store the column at 2-8°C in a refrigerator.

    **DO NOT FREEZE THE COLUMN.**

14. Concentrate the flow through and bound protein samples with the Amicon Ultra-4 centrifugal filter unit, 5,000 molecular weight limit according to the protein concentration instructions as provided by the manufacturer.

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**NOTE:** For further fractionation using the ProteomeLab PF 2D, continue with the next 2 steps.

15. After concentrating the flow-through and/or the stripped protein sample to a minimum volume, add ProteomeLab PF 2D Start Buffer to a final volume of 4.0 mL and concentrate again.
16. Finally, add a volume of Start Buffer to bring the final volume to 2.0 mL for injection on the ProteomeLab PF 2D.

It is suggested that 1-5 mg of protein be injected on the ProteomeLab PF 2D, which will require 5 to 25 cycles of the LC2 column.

## Expected Performance

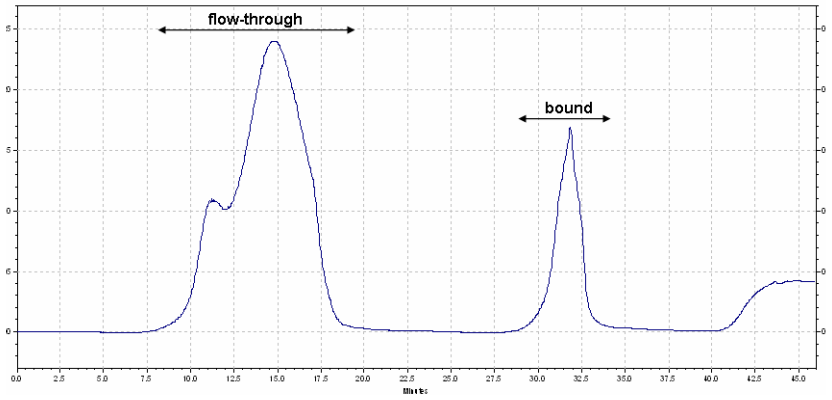


**Figure 3: Chromatography of Mouse Plasma using ProteomeLab IgY-R7 LC2.** 20 µL of mouse plasma was partitioned on the column. Note that the $A_{280}$ absorbance of the flow-through peaks appears to be greater than the bound peak. The expected volume of the flow-through fraction is 2.5 - 3.0 mL. The expected volume of the bound fraction is 3.5 - 4.5 mL.

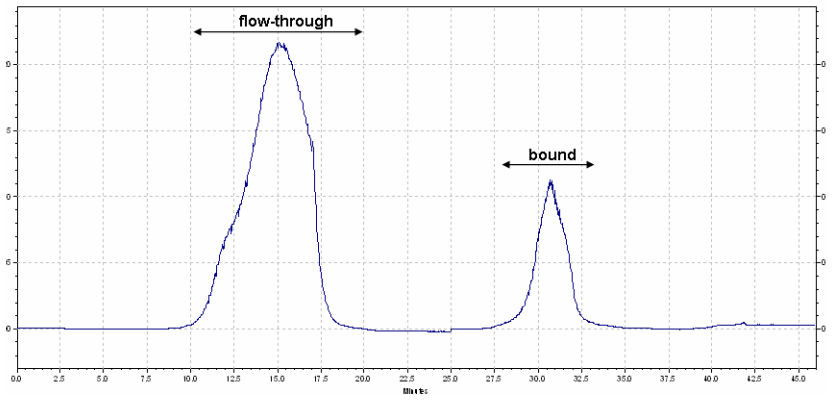


**Figure 4: Chromatography of Rat Plasma using ProteomeLab IgY-R7 LC2.** 40 µL of rat plasma was partitioned on the IgY-R7 LC2 column.

# ProteomeLab IgY-R7 LC10 Proteome Partitioning Kit

## Using these Instructions

Both of the ProteomeLab LC Proteome Partitioning Kits have specific measurements, requirements, and protocols. After noting the Protocol for this kit, See “ProteomeLab LC Partitioning Kit General Documentation” on page 21, for Troubleshooting and Recommendations.

**Reorder number: A25408**

**Observed Capacity*: 100 µL mouse serum or plasma (per run)**
**200 µL rat serum or plasma (per run)**

| Kit Contents | Product Description |
|---|---|
| IgY-R7 LC10<br><br>12.7 × 79.0 mm, Affinity Column | LC Column consisting of anti-rat serum albumin, anti-IgG, anti-fibrinogen, anti-transferrin, anti-α1-antitrypsin, anti-haptoglobin, and anti-IgM. Affinity-purified anti-IgY antibodies are covalently conjugated through their Fc portion to 60 µm polymeric microbeads as a 50% resin in Dilution Buffer with 0.02% Sodium Azide. |
| 10x Dilution Buffer (3 x 220 mL) | Tris Buffered Saline: 100 mM Tris-HCl, pH 7.4, 1.5 M NaCl. For sample dilution, washing and equilibrating column. |
| 10x Stripping Buffer (2 x 200 mL) | 1 M Glycine-HCl, pH 2.5 for stripping off bound proteins from column. |
| 10x Neutralization Buffer (2 x 250 mL) | 1 M Tris-HCl, pH 8.0 for neutralizing column and eluted proteins. |
| 100 Spin Filters, 0.45 µm | For sample clean up before loading column to remove sample particulates and extend column life. |

*The IgY Microbeads are reusable for 100 times under proper conditions.

**IMPORTANT: All buffers are shipped as 10x buffer. Dilute buffers to 1x with HPLC grade water prior to use. For neutralization of eluted proteins, retain 200 mL of undiluted 10x Neutralization Buffer.**

# ProteomeLab LC Column Common Characteristics

| | |
|---|---|
| Antibody Produced From: | Chicken, polyclonal. Antigen affinity-purified |
| Column Storage Buffer: | Dilution Buffer with 0.02% sodium azide |
| Column Storage Instruction: | 2-8°C<br>**Do not freeze.** |
| Recommended Application: | Plasma or serum protein separation, immunoprecipitation. |

## Column Specifications

| | |
|---|---|
| Column Name: | IgY-R7 LC10 |
| Column Size: | 12.7 x 79.0 mm (10 mL bed volume) |
| Column body materials: | Polycarbonate column cylinder, Polyethylene frit, Tefzel caps, Buna-N-rubber O rings, Delrin nut fittings, ETFE ferrules, and Teflon PFA tubing. |
| Column capacity: | 100 µL mouse serum/plasma<br>200 µL rat serum/plasma |
| Maximum pressure: | 100 PSI (6 bar) |
| Operating temperature: | 18-25° C |
| Column packing material: | Antibody-modified resin |
| Immobilized ligands: | Affinity-purified IgY polyclonal antibodies to rat serum albumin, IgG, fibrinogen, transferrin, α1-antitrypsin, haptoglobin, and IgM. |
| Flow rate range: | 0.5-2 mL/min |

## Materials Required but Not Provided by Beckman Coulter

- ProteomeLab SP Sample Preparation System, including one F2402H Fixed Angle rotor and one SX4250 Swinging Bucket rotor with Adapter PN 392258 for 50 mL conical tube
- ProteomeLab PPS Proteome Partitioning System
- Pipette and pipette tips (1-200 µL and 200-1000 µL)
- Column Storage Dilution Buffer with 0.02% (w/v) Sodium Azide
- Amicon Ultra-15 Centrifugal Filter Device (Millipore, Catalog No. UFC9 005 08 for the 8 pack, Catalog No. UFC9 005 24 for the 24 pack, or Catalog No. UFC9 005 96 for the 96 pack)
- HPLC grade water
- 50 mL polypropylene tubes for fraction collection
- 1000 mL graduated cylinder
- Gloves and protective eyewear

## Safety Issues

When preparing biological samples using ProteomeLab Columns, follow general guidelines for handling biological materials and wear the appropriate protective attire.

## Methods

### Protocol for 12.7 x 79.0 mm LC10 Column (Column capacity: 100 µL mouse or 200 µL rat serum/plasma)

1. Dilute 100 ml of each stock 10x buffer with 900 ml HPLC grade water.
2. Transfer each 1x buffer to a separate 1 liter solvent bottle.
3. Before every run, ensure all buffer bottles are free of turbidity and precipitation. Replace them if these conditions are present.
4. Prime the solvent lines with the three 1x diluted buffers (Dilution, Stripping and Neutralization) according to the ProteomeLab PPS Operators Manual or the instructions of other chromatographic systems.
5. Start the 32 Karat software and select the `IgYLC10.met` method file on the ProteomeLab PPS according to the User's Guide, or program the chromatographic system, using the instructions found in the programming guidelines.
6. Set up the LC timetable. See "Appendix B: IgY-R7 LC10" on page 24 for guidelines on non-ProteomeLab PPS Systems.
7. Attach the column and set the injector to the inject position.

8. Before the first run of the day, after removing the column from the refrigerator, equilibrate the 1x Dilution Buffer for 30 min. at a flow rate of 2.0 mL/min at room temperature to ensure a flat baseline.
   - The first time the column is used and after temperature equilibration, perform two complete buffer runs without injecting a sample.
9. Dilute the 100 µl mouse sample in 500 µL of 1x Dilution Buffer to get a final volume of 600 µL (1 to 6 dilution), or 200 µL rat sample in 400 µL of Dilution Buffer (1 to 3 dilution).
10. Remove any sample particulates using a 0.45 µm spin filter. Centrifuge for 1 minute at 9200 x *g*.
11. Inject 600 µL of the diluted sample and start the separation method.
12. Collect flow-through and eluted fractions. Add 1/10 volume 10x Neutralization Buffer (1.6-1.8 mL) to eluted fraction (16-18 mL). Store both fractions at -70°C if they are not analyzed immediately.
13. If the column is not going to be reused within 7 days, equilibrate the column with 1x Dilution Buffer that contains 0.02% (w/v) sodium azide. Start the 32 Karat software and select `IgYLC10_store.met` method file on the ProteomeLab PPS System, or pump the 1x Dilution Buffer with 0.02% sodium azide at a flow rate of 1.0 mL/min. for 20 min. through the column on other chromatographic systems. Store the column at 2-8°C in a refrigerator.

    **DO NOT FREEZE THE COLUMN.**

14. Concentrate the flow through and bound protein samples with the Amicon Ultra-15 centrifugal filter unit, 5,000 molecular weight limit according to the protein concentration instructions as provided by the manufacturer.

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**NOTE:** For further fractionation using the ProteomeLab PF 2D, continue with the next 2 steps.

15. After concentrating the flow-through and/or the stripped protein sample to a minimum volume, add ProteomeLab PF 2D Start Buffer to a final volume of 4.0 mL and concentrate again.
16. Finally, add a volume of Start Buffer to bring the final volume to 2.0 mL for injection on the ProteomeLab PF 2D.

It is suggested that 1-5 mg of protein be injected on the ProteomeLab PF 2D, which will require 1 to 5 cycles of the LC10 column.

## Expected Performance

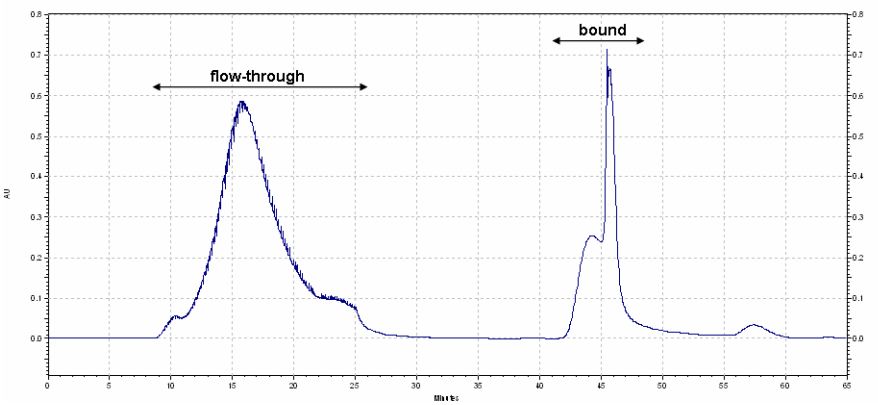


**Figure 5: Chromatography of Mouse Plasma/Serum using ProteomeLab IgY-R7 LC10.** 135 µL of mouse plasma was partitioned on the column. Note that the $A_{280}$ absorbance of the flow-through peak appears to be greater than the bound peak. The expected volume of the flow-through fraction is 9-11 mL. The expected volume of the bound fraction is 16-18 mL.

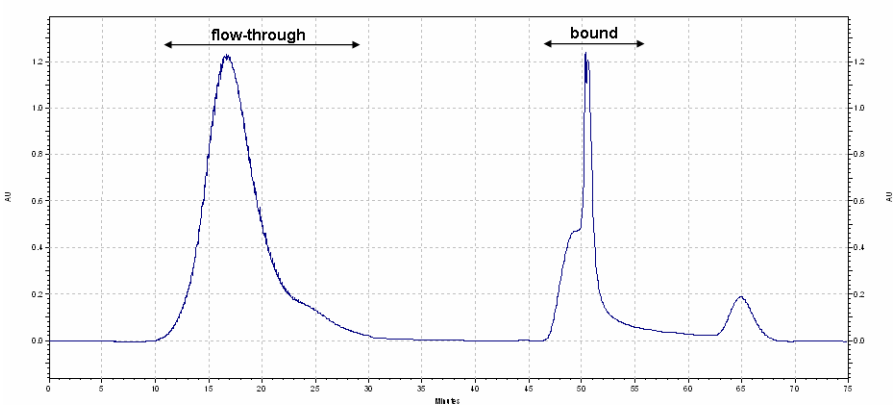


**Figure 6: Chromatography of Rat Plasma using ProteomeLab IgY-R7 LC10.** 200 µL of rat plasma was partitioned on the column.

## Troubleshooting

| Symptom | Possible Cause | Corrective Action |
|---|---|---|
| Reduction in protein binding with abnormal capacity peak height | • Column may have not been regenerated well enough from previous runs.<br>• Buffers contaminated with bacteria or fungi. | • Elute bound proteins with Stripping Buffer for 2 additional column volumes, and then immediately neutralize and re-equilibrate the column with 1x Neutralization Buffer and Dilution Buffer.<br>• Carefully check for signs of biological growth and precipitates in the Buffers. Replace with fresh Buffers for optimized column performance. |
| High Backpressure or Distorted peak shape | Clogged inlet frits | Remove particulates from all samples with a 0.45 µm spin filter before loading. Failure to pre-filter the sample can diminish the column lifetime. |
| No bound fraction peak | • Insufficient elution with Stripping Buffer. Bound proteins should only be removed from the column using Stripping Buffer.<br>• Column may not have been regenerated well enough from previous runs, resulting in lost capacity.<br>• Buffers contaminated with bacteria or fungi. | • Check LC timetable to ensure enough column time exposure to Stripping Buffer for complete removal of bound proteins.<br>• To correct this, elute bound proteins with Stripping Buffer for 2 additional column volumes, and then neutralize and re-equilibrate the column with Neutralizing Buffer and Dilution Buffer.<br>• Check for signs of biological growth in the reservoirs. Replace with fresh buffers for optimized column performance. |

For Beckman Coulter contact information, see “Additional Technical Support” on page 7.

## Recommendations

- **Sample dilution**
  It is not recommended to load crude serum directly onto the column. Follow instructions for plasma or serum dilution (five times dilution with Dilution Buffer).
- **Column performance**
  **Adjust LC timetable based on your own instrument if necessary.** Do not expose the columns to solvents other than the three provided buffers (Dilution, Stripping, and Neutralization).
- **Column storage**
  Always store the columns after equilibrating with Dilution Buffer in a refrigerator at 2-8°C when not in use to minimize loss in column capacity. Dilution Buffer should contain 0.02% (w/v) sodium azide for prolonged column storage greater than 7 days.
- **Lyophilization of flow-through fractions**
  Buffer exchange to a volatile buffer (for example, ammonium bicarbonate) is recommended prior to lyophilization due to high salt concentration in Dilution Buffer.

**Caution: Do not expose columns to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, or reducing agents, and other protein denaturing agents.**

**Warning: FOR RESEARCH USE ONLY - this product is NOT TO BE USED AS AN *IN-VITRO* DIAGNOSTIC.**

**Storage: Store the affinity column at 2-8°C upon receiving and when not in use. DO NOT FREEZE THE COLUMN!**

# Appendix A: IgY-R7 LC2

**Table 1:** LC Method for 6.4 x 63 mm LC2 Column

The table below provides a starting point for developing a method file for use with the LC2 Column. The detector wavelength should be set to 280 nm and 1 Hz data collection rate.

| Cycle | Time (min) | Dilution Buffer | Stripping Buffer | Neutralization Buffer | Flow rate (mL/min) |
|---|---|---|---|---|---|
| Injection | | | | | |
| Wash | 0 | 100 | 0 | 0 | 0.1 |
| Wash | 10.01 | 100 | 0 | 0 | 0.2 |
| Wash | 17.01 | 100 | 0 | 0 | 1.0 |
| Stripping | 22.01 | 0 | 100 | 0 | 1.0 |
| Neutralization | 36.01 | 0 | 0 | 100 | 1.0 |
| Re-equilibrium | 42.01 | 100 | 0 | 0 | 1.0 |
| Stop | 48.00 | | | | |

# Appendix B: IgY-R7 LC10

**Table 2:** LC Method for 12.7 × 79.0 mm LC10 Column

The table below provides a starting point for developing a method file for use with non-Beckman Coulter LC Systems. The detector wavelength should be set to 280 nm and 1 Hz data collection rate.

| Cycle | Time (min) | Dilution Buffer | Stripping Buffer | Neutralization Buffer | Flow rate (mL/min) |
|---|---|---|---|---|---|
| Injection | | | | | |
| Wash | 0 | 100 | 0 | 0 | 0.5 |
| Wash | 25.01 | 100 | 0 | 0 | 2.0 |
| Stripping | 30.01 | 0 | 100 | 0 | 2.0 |
| Neutralization | 48.01 | 0 | 0 | 100 | 2.0 |
| Re-equilibrium | 56.01 | 100 | 0 | 0 | 2.0 |
| Stop | 65.00 | | | | |

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