

BioLogic DuoFlow™ Valving Guide

Valving Schemes for the BioLogic DuoFlow Chromatography System



BioLogic DuoFlow Valving Guide

Introduction

The BioLogic DuoFlow Chromatography System is a powerful and versatile biocompatible chromatography system for the purification of proteins, peptides, nucleic acids, and other small molecules.

The modular nature of the BioLogic DuoFlow System makes it very easy to upgrade the gradient pump and to add peripheral devices such as BioLogic Maximizer™ Valve System Detectors, auxiliary sample loading pumps, and fraction collectors. In addition, the unique versatility of the BioLogic DuoFlow System is enhanced by the availability of several different valves for automating both routine and more complex separation protocols. For the user, this means reliable operation and reproducible results, run after run, year after year.

This guide describes typical valving applications. The applications range from use of a single AVR7-3 Sample Injection Valve to simply equilibrate a column, inject sample, and elute the column, to multidimensional chromatography by using a combination of AVR7-3, AVR9-8, SV5-4, and a BioLogic Maximizer Valve System to connect multiple columns for multistep purifications. BioLogic DuoFlow Software makes working with valves easy and intuitive. The software automatically senses each valve and displays it in a graphical format on the Manual screen. The Setup Editor makes it simple to program any valving scheme by its “valve aliasing” feature and its ability to save an unlimited number of user-identified valving configurations.






The “Make-Before-Break” Advantage

To accommodate the wide range of pressure and flow rates of the BioLogic DuoFlow System, the AVR7-3 and AVR9-8 Valves use a patented make-before-break design that prevents pressure spikes when the valves rotate from one port to another. This is beneficial when using fragile columns or flow-sensitive detectors and prevents pump shutdowns due to transient overpressure situations.

Reliability Guaranteed

Reliability is a vital prerequisite of any valve, especially when used with the wide range of buffers, salt solutions, and solvents seen in today’s laboratory. Thousands of Bio-Rad chromatography valves are in daily use worldwide, handling everything from 1.0 M salt solutions to 100% acetonitrile. All valves are completely biocompatible with no metallic parts in contact with the liquid flow.

BioLogic DuoFlow System Valve Selection Guide

Valve*	Type	Ports	
SV5-4	Low-pressure solenoid valve for stream selection	Common, 1, 2, 3, 4	
SVT3-2	Low-pressure solenoid valve for stream selection or flow diversion	Common, 1, 2	
AVR9-8	High-pressure make-before-break stream-select valve	Common, 1–8	
AVR7-3	High-pressure make-before-break injection valve	1–7 (Load, Inject, and Purge, 3 positions)	
BioLogic Maximizer Valve System	Enables buffer blending, double flow rates, and twice the valving capacity of the standard BioLogic DuoFlow System		

* Up to three SV and three AVR valves can be connected to the BioLogic DuoFlow System. Up to six SV and six AVR valves can be connected to the BioLogic DuoFlow Maximizer™ and BioLogic DuoFlow Pathfinder™ Systems.

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<p>2 Multiple Buffer Selection</p> <p>Gradient pump inlet valves may be used to select a different buffer, to add sanitation or storage buffer at the end of a run, or to load sample directly through the gradient pump.</p>	<p>AVR7-3 and 2 x AVR9-8*</p> <p>* Or 2 x SV5-4 or SVT3-2</p>	7
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Application	Valves Needed	Page
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<p>11 Flowthrough Profiling Using Three AVR7-3 Valves A sample is loaded onto one column and nonbinding components (flow-through proteins) pass through up to two other columns. Each column may then be eluted individually. Gradient pump inlet valves are used if different buffers are needed for equilibration and elution of each column.</p>	<p>3 x AVR7-3 Up to 2 x SV5-4 are optional for buffer selection</p>	18
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Application 1



Basic AVR7-3 Sample Injection Valve Operation Using Load, Inject, and Purge Positions

Load position is for filling a loop with sample or for running buffer through a column; Inject position is for injecting contents of the sample loop onto a column; Purge position is for priming the BioLogic DuoFlow Workstation Gradient Pump and purging lines to waste without removing the column from the system.

AVR7-3 in Load Position (Figure A)

The column may be equilibrated or eluted. Sample is loaded into the loop. If the loop is overfilled, the sample runs to waste. Never remove the syringe before the sample is injected onto the column.

AVR7-3 in Inject Position (Figure B)

The sample is injected onto the column.

AVR7-3 in Purge Position (Figure C)

Flow from the gradient pump goes directly to waste, bypassing the column.

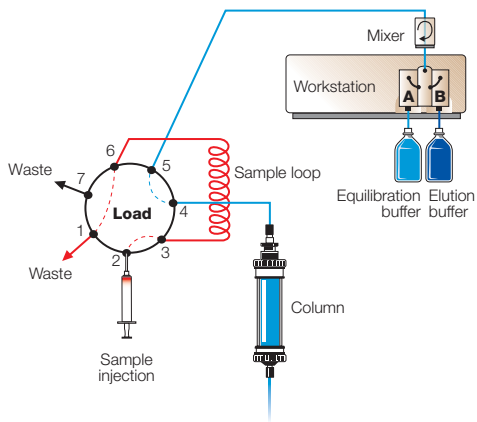


Fig. A

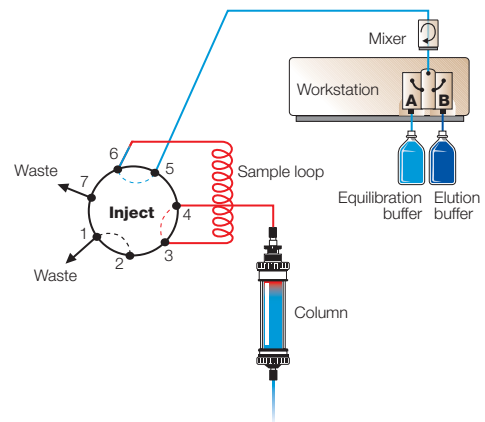


Fig. B

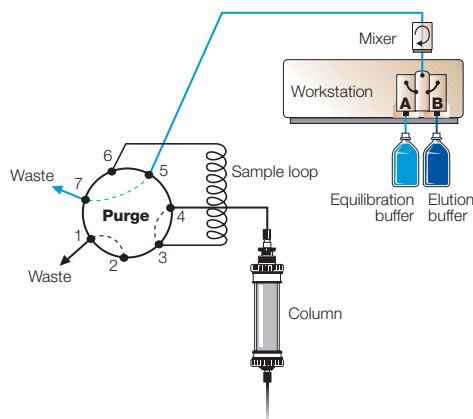
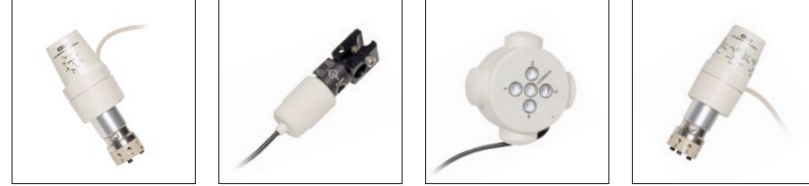


Fig. C

Application 2

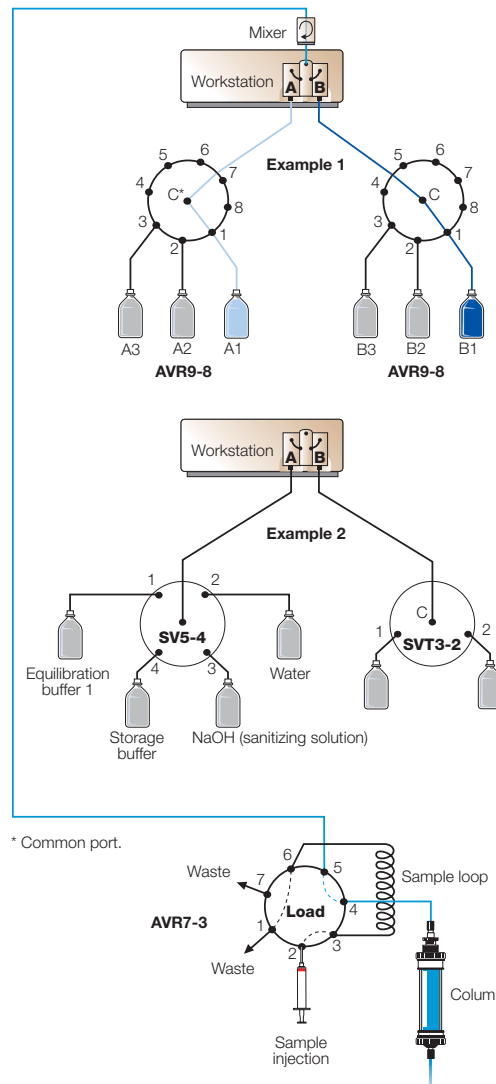


Multiple Buffer Selection

Gradient pump inlet valves may be used to select a different buffer, to add sanitation or storage buffer at the end of a run, or to load sample directly through the gradient pump.

Any valve may be attached to gradient pump inlets A or B. Two examples are shown.

Use the Manual screen valve control to switch between valve ports and to prime each valve position using a syringe before running a method.



Application 3



Reverse-Flow Chromatography for Affinity Purifications

This is especially useful when purifying antibodies using protein A or other affinity columns. The protein is bound at the top of the column and nonbinding material is washed out. Flow to the column is then reversed and the bound proteins elute from the top of the column in very concentrated form, which helps prevent denaturation.

Step 1 (Figure A)

Sample has first been loaded into the loop of valve 1 in Load position. Valve 1 moves to Inject position and the sample passes to valve 2. With valve 2 in Inject position, sample is pumped onto the top of the column and nonbinding material is washed out of the column (top to bottom).

Step 2 (Figure B)

Note that ports 1 and 2 of valve 2 have been connected using a short piece of tubing. Valve 1 moves to Load position for column washing and elution. The loop is now out of the flow path. With valve 2 in Load position, the column is eluted by reverse flow (bottom to top).

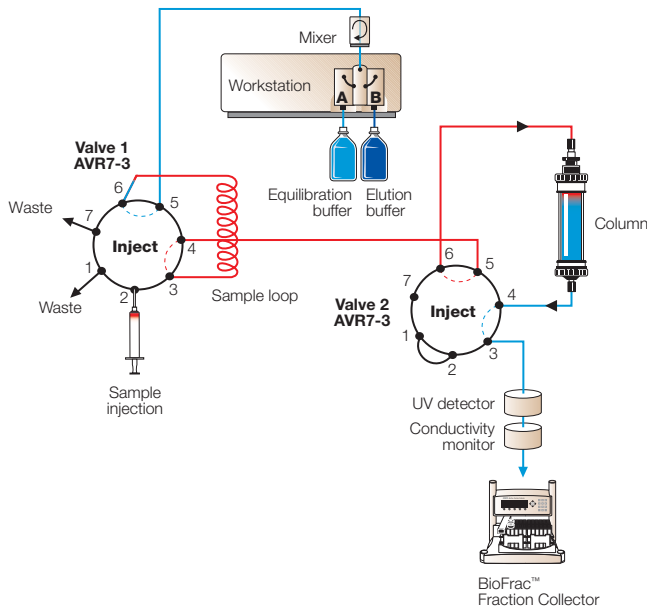


Fig. A

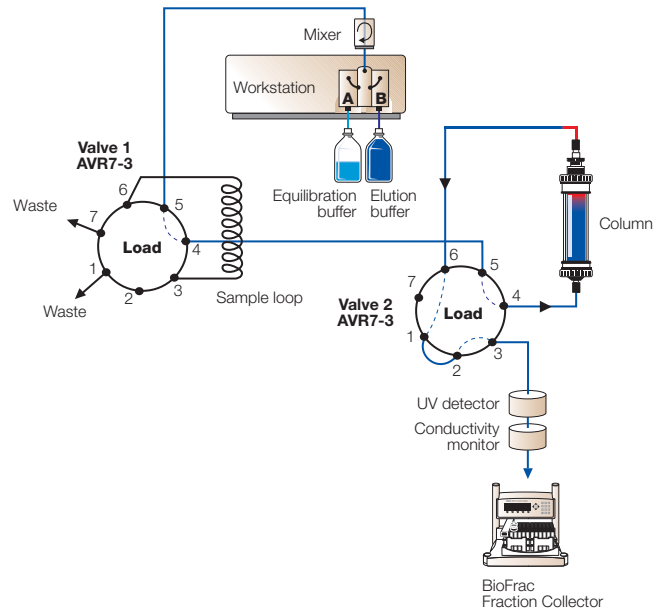


Fig. B

Application 4



Using an AVR7-3 Valve for Two-Column Switching

Test two columns side by side and compare their separation characteristics.

Flow-through Column 1 (Figure A)

Two columns are connected to valve 2 as shown. With valve 2 in Load position, flow from the gradient pump goes to column 1.

Flow-through Column 2 (Figure B)

With valve 2 in Inject position, flow from the gradient pump goes to column 2.

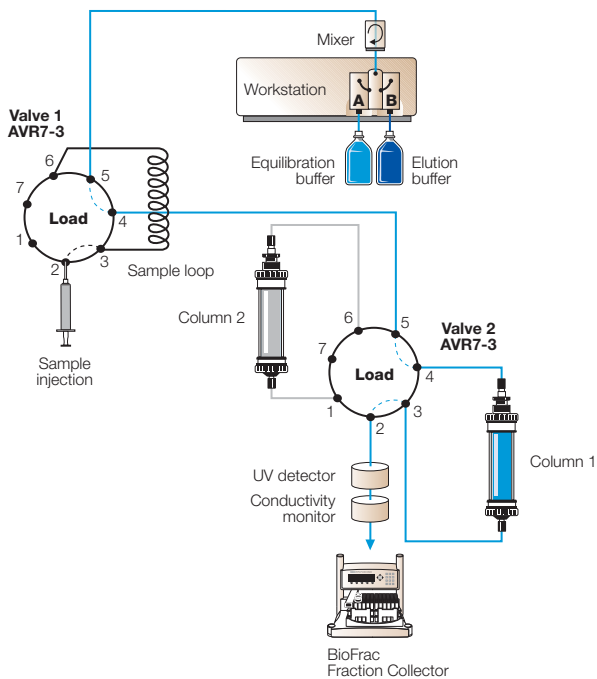


Fig. A

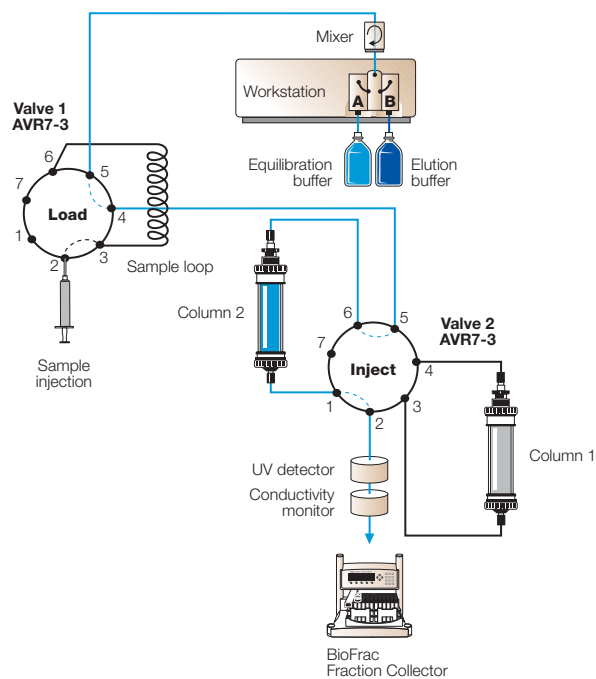


Fig. B



Two-Column Switching With Buffer Selection (Figure C)

Additional gradient pump inlet valves for multiple buffer selection may also be used with this application.

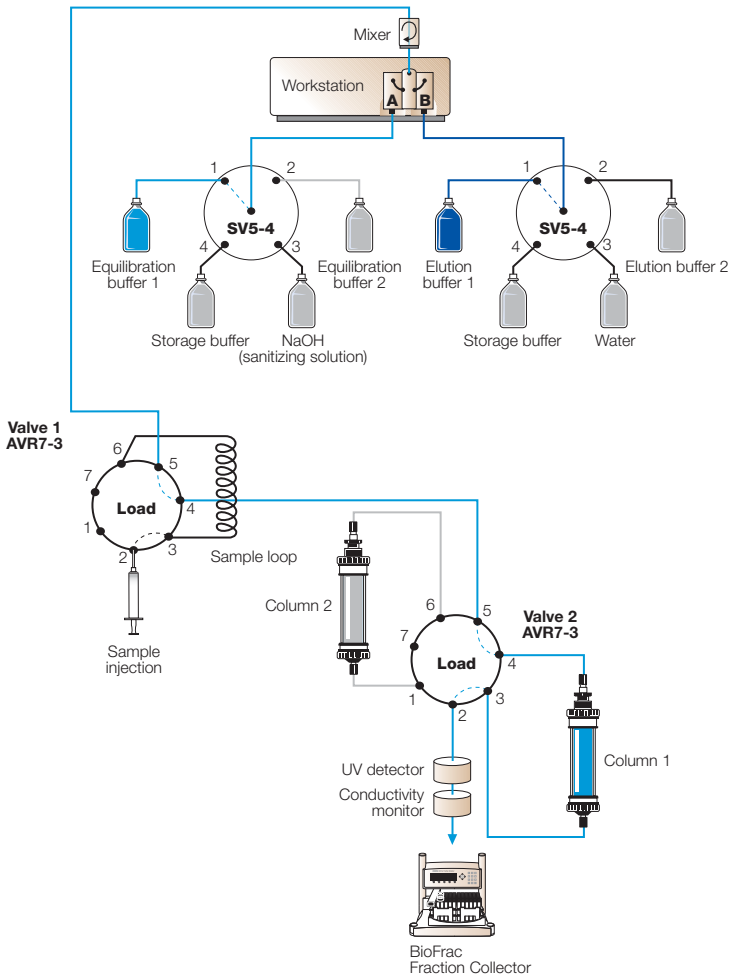


Fig. C

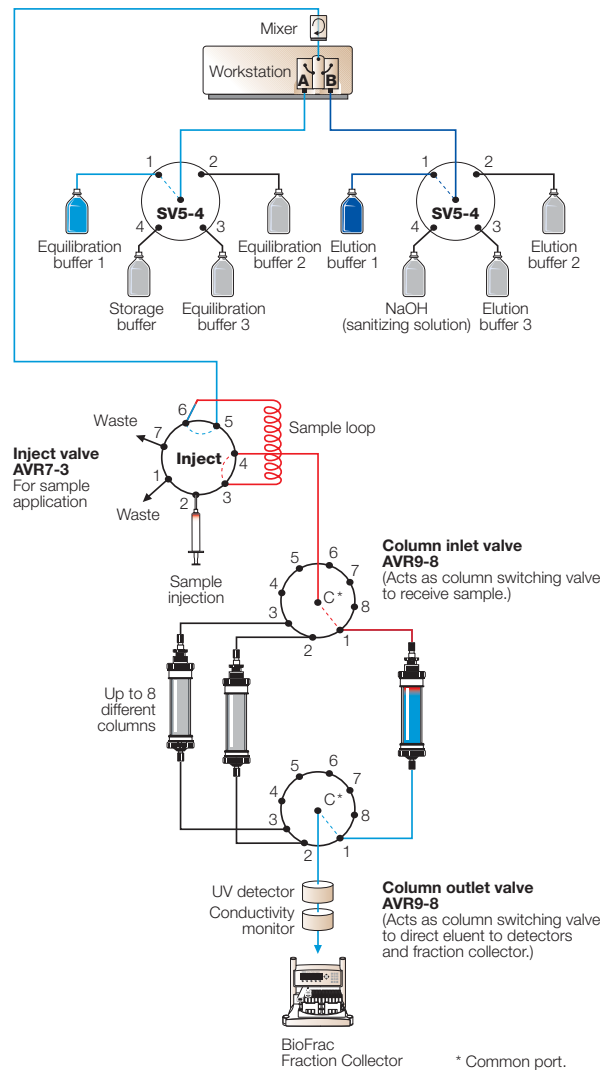
Application 5



Using Two AVR9-8 Valves for Eight-Column Switching

Test up to eight columns side by side and compare their separation characteristics. Additional gradient pump inlet valves for multiple buffer selection typically are used with this application.

Two AVR9-8 Valves act as column inlet and outlet valves to determine which column is in line. SV5-4 Valves act as gradient pump inlet valves for buffer selection. When AVR7-3 Valve 1 is in Inject position, sample is injected onto the selected column.

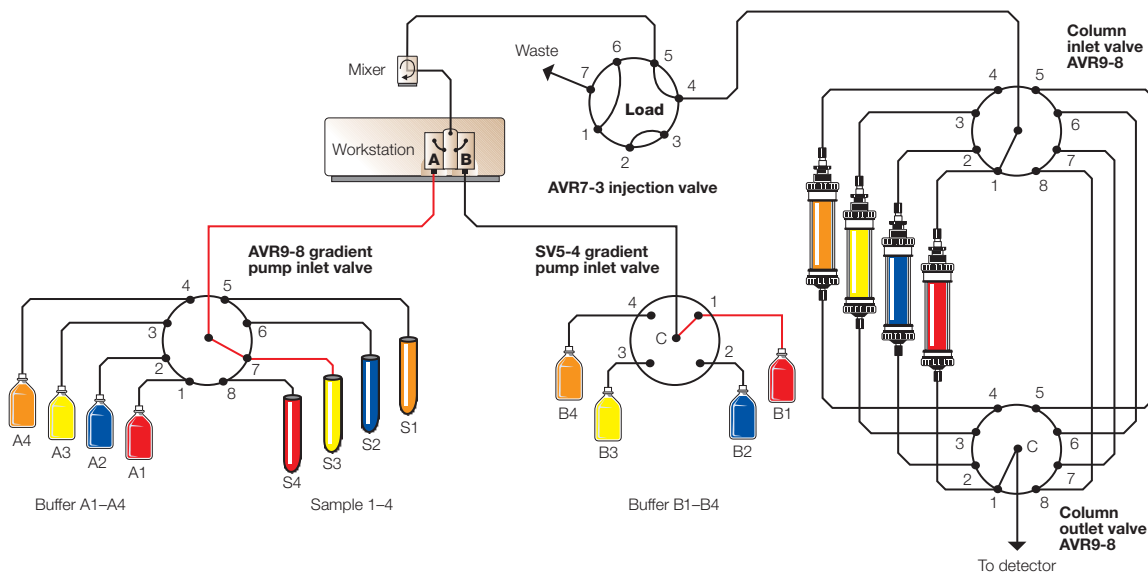


Application 6



Mini Autosampler for Multiple Sample Injection

SV5-4 and AVR9-8 Valves can be used as a mini autosampler to direct load samples through the gradient pump when connected to a BioLogic DuoFlow System. In this configuration, up to 7 samples can be loaded. One port must be plumbed to a rinse solution in order to rinse the valve and tubing between sample injections. The example below shows a plumbing configuration that can be used to load 4 samples with 8 different buffer systems on 4 different columns. These runs could be used for scouting experiments that scout on sample preparation, or alternatively for purification of a variety of samples as part of a queue in conjunction with a column switching valve.



Application 7



Mini Autosampler With Automated Loop Fill and Rinse

Use an auxiliary pump and a stream-select valve as a four- or eight-place mini autosampler. Combine a peristaltic pump, such as the Model EP-1 Econo Pump or Econo Gradient Pump, with an inlet valve for automated filling and rinsing of a sample loop.

Step 1

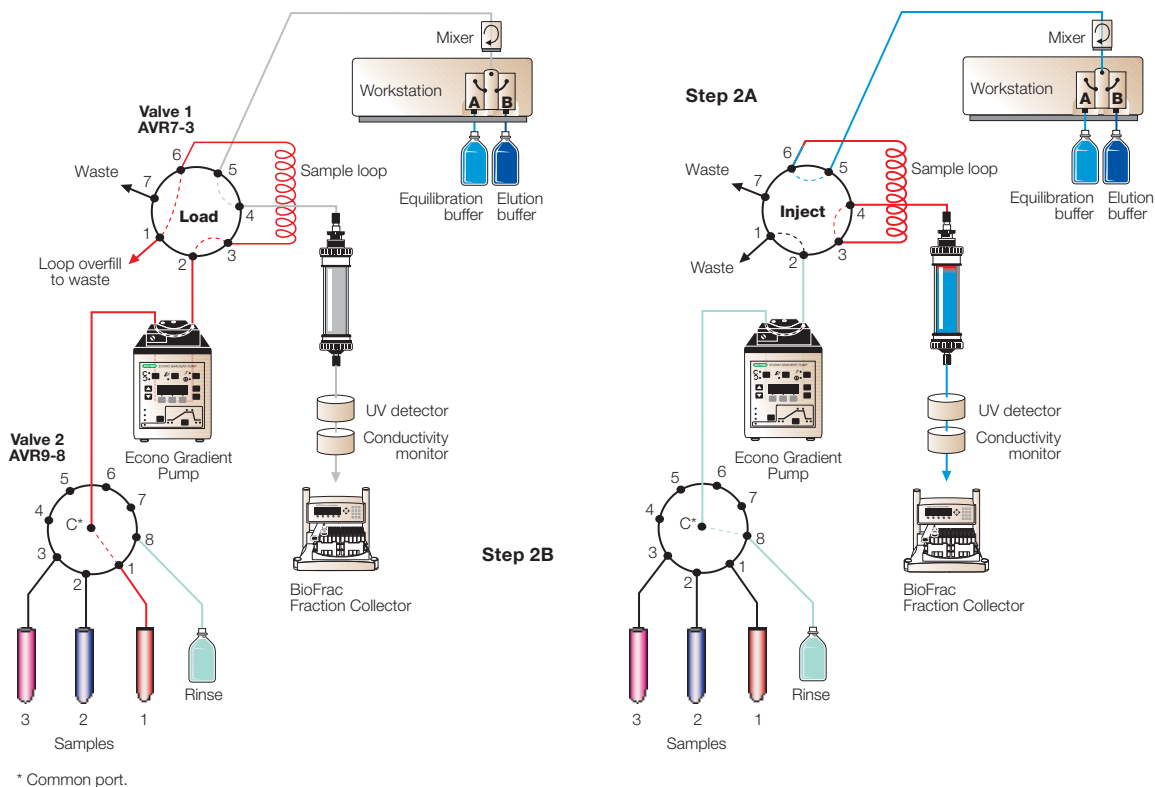
Valve 1 is in Load position for sample loading into the loop. Valve 2 is an AVR9-8 Valve for sample and rinse selection. The BioLogic DuoFlow Software automatically loads the selected sample into the loop.

Step 2A

Valve 1 moves to Inject position and the sample is injected onto the column.

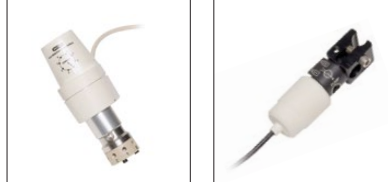
Step 2B

When sample injection is complete, valve 1 is automatically returned to Load position. The software can then automatically select a defined rinse position and rinse the loop prior to another sample injection.



Step 1

Application 8



Large-Volume Sample Injection Onto a Low Backpressure Column

Use an auxiliary pump and a valve such as the SVT3-2 for direct injection of sample onto a column. This application uses a peristaltic pump such as the Model EP-1 Econo Pump or the Econo Gradient Pump to load large amounts of crude sample onto a column for a first-step separation. A 40 psi backpressure device may be placed after the workstation gradient pump to improve pump performance. Additional pump inlet valves for multiple sample selection, rinsing, and buffer selection may also be used with this application.

Step 1

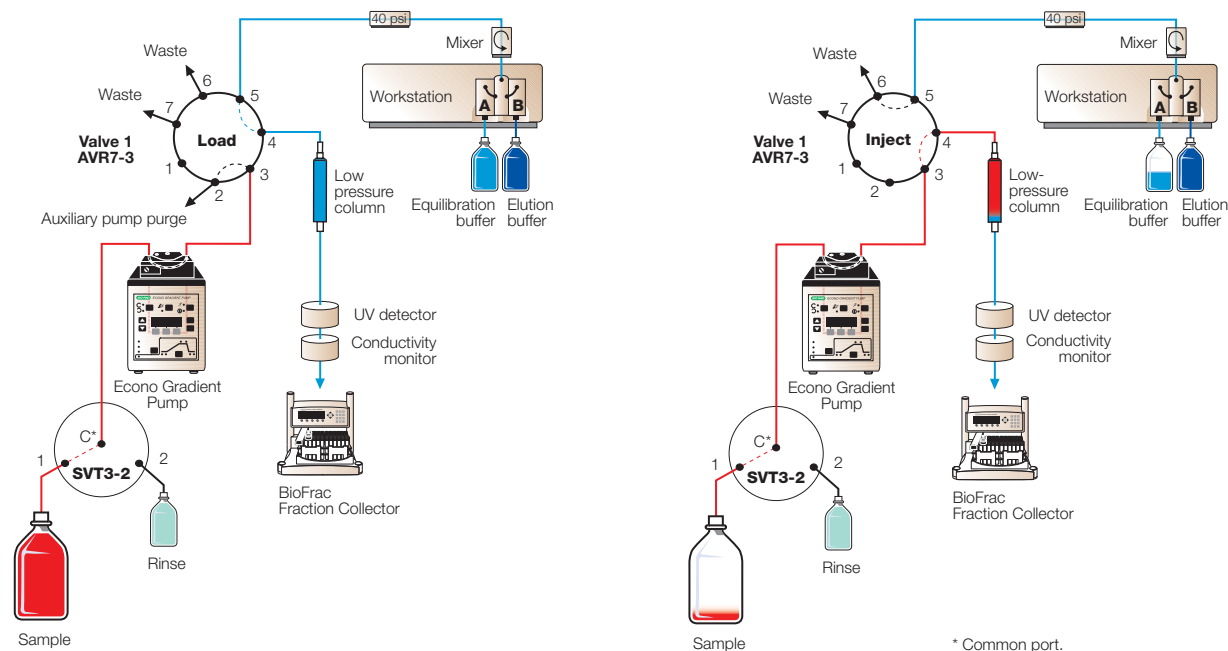
Valve 1 is in Load position and the column is equilibrated. The auxiliary pump is primed with sample.

Step 2A

Valve 1 moves to Inject position and the sample is pumped directly onto the column by the auxiliary pump. The BioLogic DuoFlow Workstation Gradient Pump stops while the auxiliary pump is injecting sample.

Step 2B (Not Shown)

When sample injection is complete, valve 1 is automatically returned to Load position and the workstation gradient pump is used to elute sample from the column.



Step 1

Step 2A

Application 9



Large-Volume Fraction Collection

The BioLogic DuoFlow System provides three mechanisms for doing large-volume fraction collection.

Example 1

Use one AVR9-8 Valve as a fraction collector to collect 8 fractions. Individual “change valve” steps are programmed in the method protocol to collect the desired fractions.

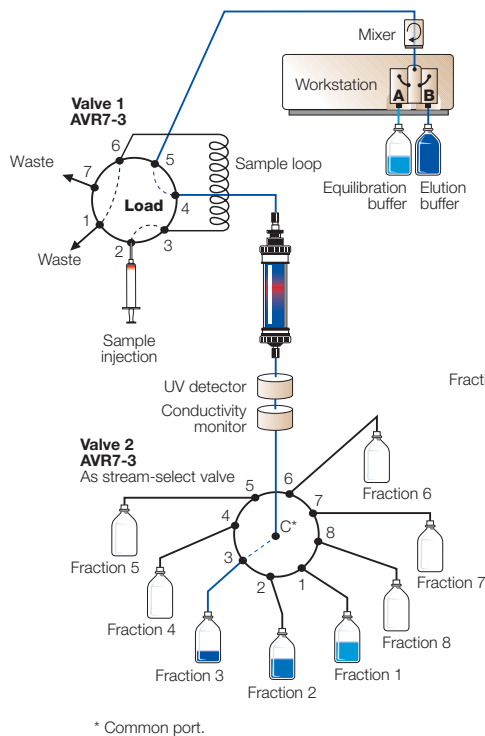
Example 2

Use two AVR9-8 Valves as a fraction collector to collect 15 fractions.

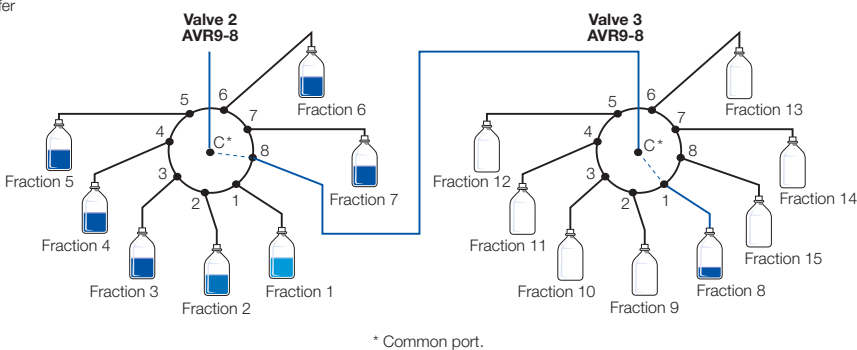
Example 3

Use the preparative fraction collector rack option for the BioFrac Fraction Collector.

The BioFrac Prep-20 Preparative Rack can be used to collect fractions in up to 20 collection vessels of any size, with milliliter to liter collection volumes. It can be used at flow rates up to 80 ml/min. Tygon Tubing 3/8” OD, 1/4” ID is typically used to plumb the Prep-20 funnels to the collection vessels.



Example 1

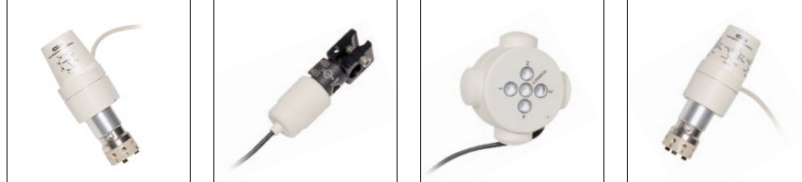


Example 2



Example 3

Application 10



Tandem Chromatography

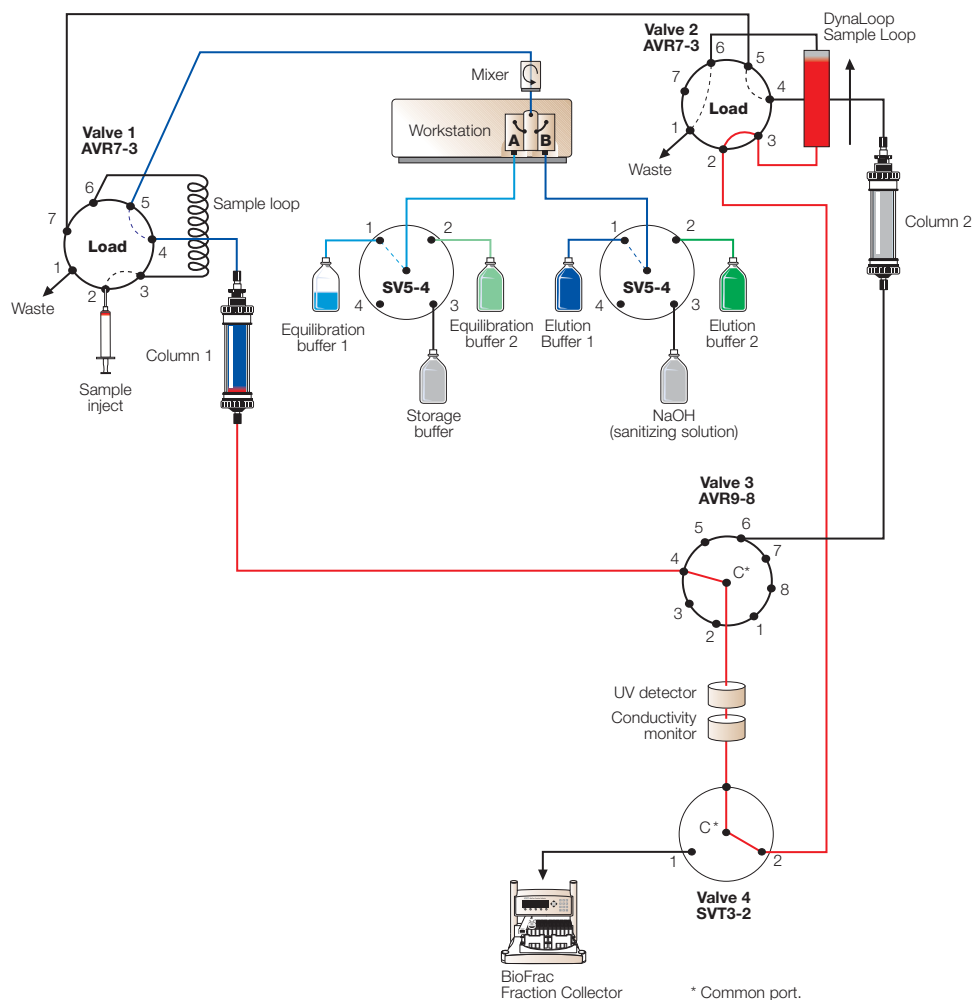
Tandem chromatography is used to automatically collect selected fractions from one column and inject them onto a second column. Eluted fractions from the first column are collected into a DynaLoop or other sample loop, which are then eluted through the second column. Gradient pump inlet valves are used if different buffers are needed for equilibration and elution of each column. For example, monoclonal antibodies can be purified with column 1 as a cation exchanger and column 2 as ceramic hydroxyapatite, at a neutral pH. This could be an alternative to affinity chromatography, which requires low-pH buffers to elute antibodies.

Step 1A (Not Shown)

The sample is loaded into the loop of valve 1 and injected onto column 1. With the appropriate buffer selected from the gradient pump inlet valves, column 1 is eluted. Valve 4 directs the flow to the fraction collector for collection or diversion to waste.

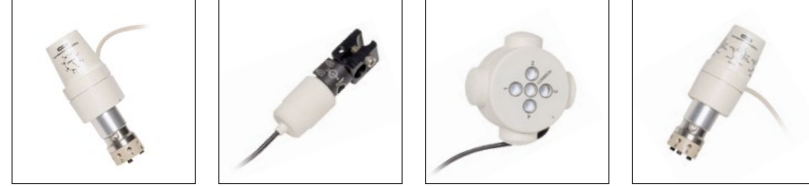
Step 1B

At a predetermined time or volume, valve 4 moves to position 2 and a portion of the column eluate is captured in the loop attached to valve 2.



Step 1B

Application 10 (continued)

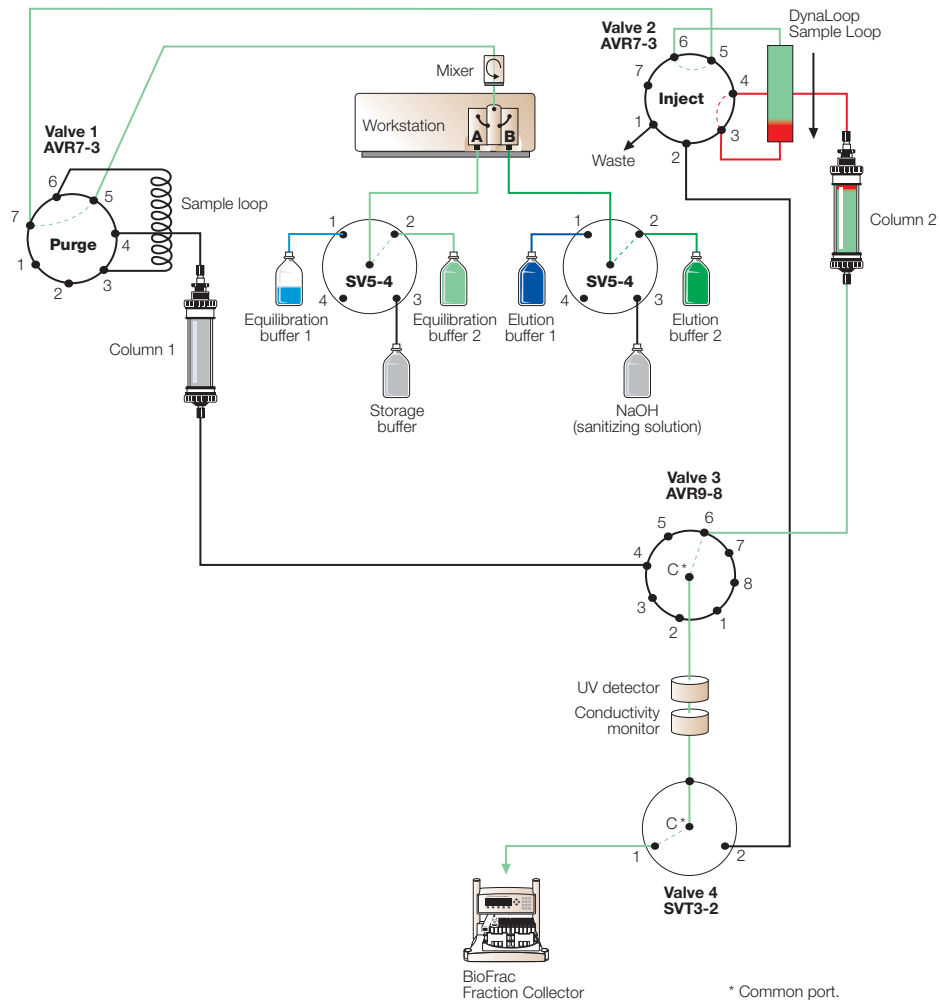


Step 2

Valve 1 moves to Purge position to put valve 2 and column 2 into the flow path. Valves 3 and 4 change position to connect column 2 to the detectors and fraction collector. With the appropriate buffer selected from the gradient pump inlet valves, the sample stored in the loop is injected onto column 2 when valve 2 moves to Inject position.

Step 3 (Not Shown)

Valve 2 reverts to Load position and column 2 is eluted.



Step 2

Application 11



Flowthrough Profiling Using Three AVR7-3 Valves

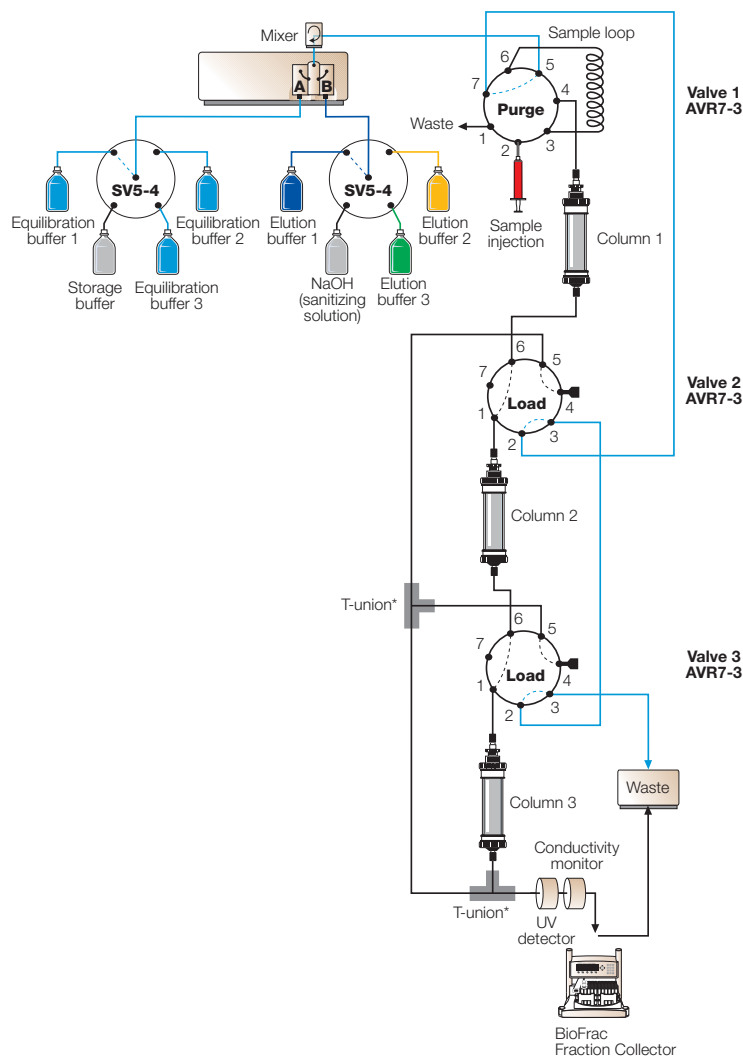
Flowthrough profiling is a technique used to capture multiple components from a mixture in a single run. Here, a mixture of proteins is loaded onto the first column. The nonbinding components of the mixture are then allowed to flow through a second and third column. Each column may then be eluted individually. The columns used in this technique are selected based on the properties of the components to be captured. SV5-4 or AVR9-8 Gradient Pump Inlet Valves may be used for selection of multiple buffers. Gradient pump inlet valves are used if different buffers are needed for equilibration and elution of each column.

Step 1

With valve 1 in Purge position and valves 2 and 3 in Load position, the gradient pump may be primed with the buffers from the gradient pump inlet valves. Two T-unions* are used to connect tubing. Port 4 of valves 2 and 3 should be end-capped to prevent misdirected flow.

To equilibrate columns individually:

Column	Valve 1	Valve 2	Valve 3
1	Load	Inject	Load or Inject
2	Purge	Inject	Inject
3	Purge	Load	Inject



Step 1

* P-12 TEE, with P-200/P-235, PEEK, available from Upchurch Scientific.

Application 11 (continued)

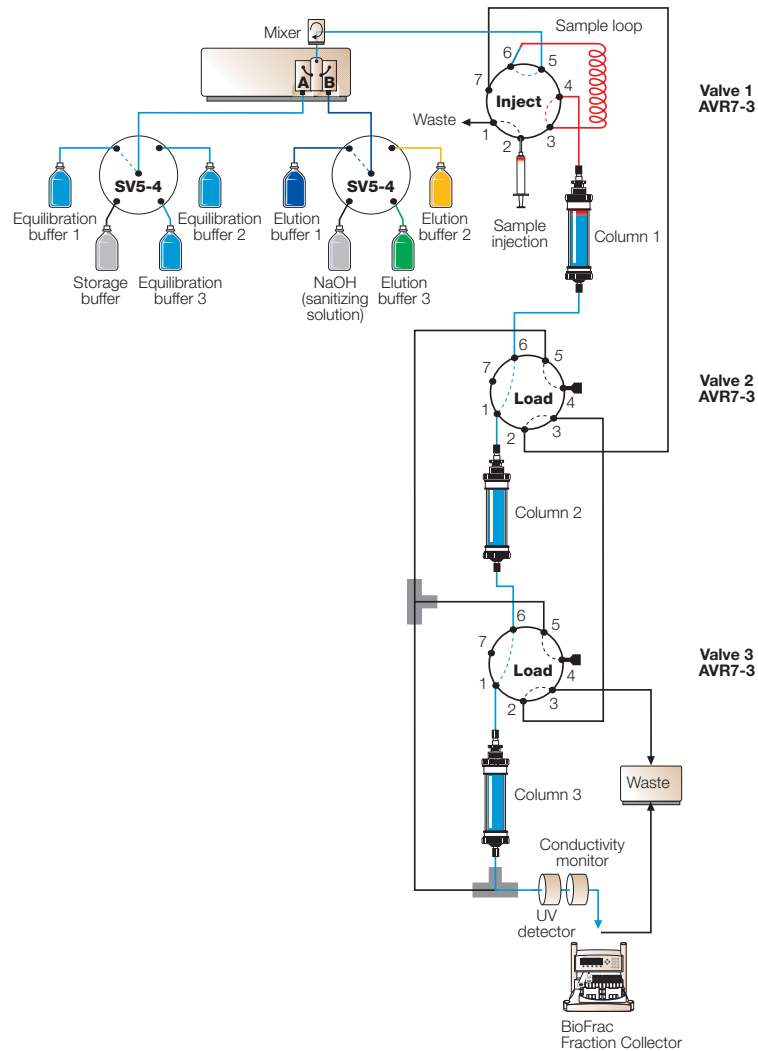


Step 2A (Not Shown)

Valve 1 is placed initially in Load position. Valves 2 and 3 remain in Load position. All three columns are equilibrated and the flow goes to waste via the fraction collector diverter valve.

Step 2B

Sample is loaded into the loop. Valve 1 is placed in Inject position and the sample is injected onto column 1. With valves 2 and 3 remaining in Load position, the unbound components of the sample pass through all three columns and then to the fraction collector (or to waste via the fraction collector diverter valve). The flow path is shown in blue.



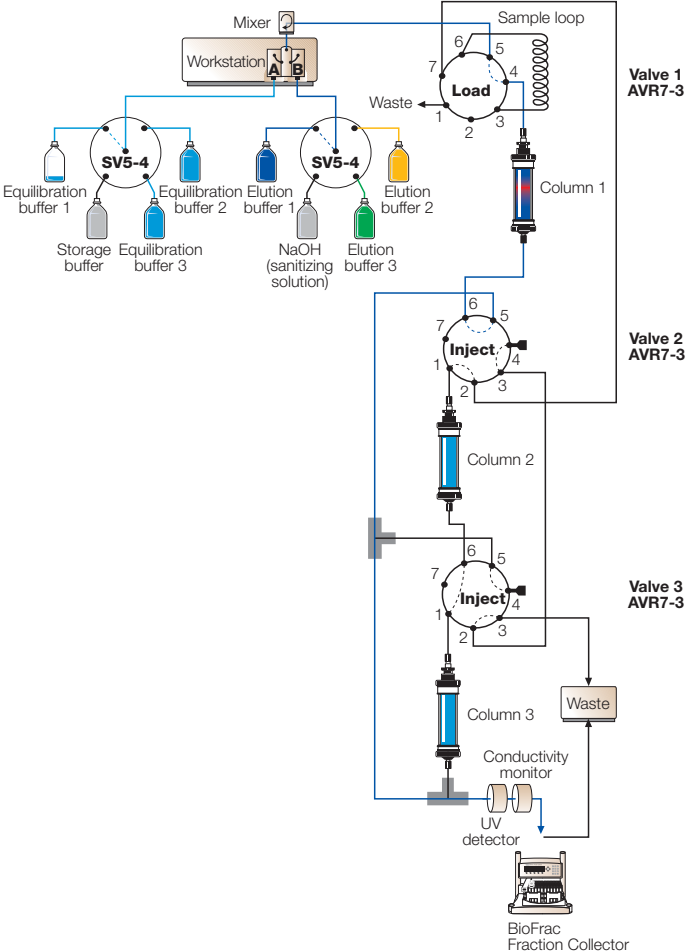
Step 2B

Application 11 (continued)



Step 3

To elute column 1, valve 1 is in Load position. Valves 2 and 3 are both in Inject position and are out of the flow path. The flow from column 1 goes to the fraction collector.



Step 3

Application 11 (continued)

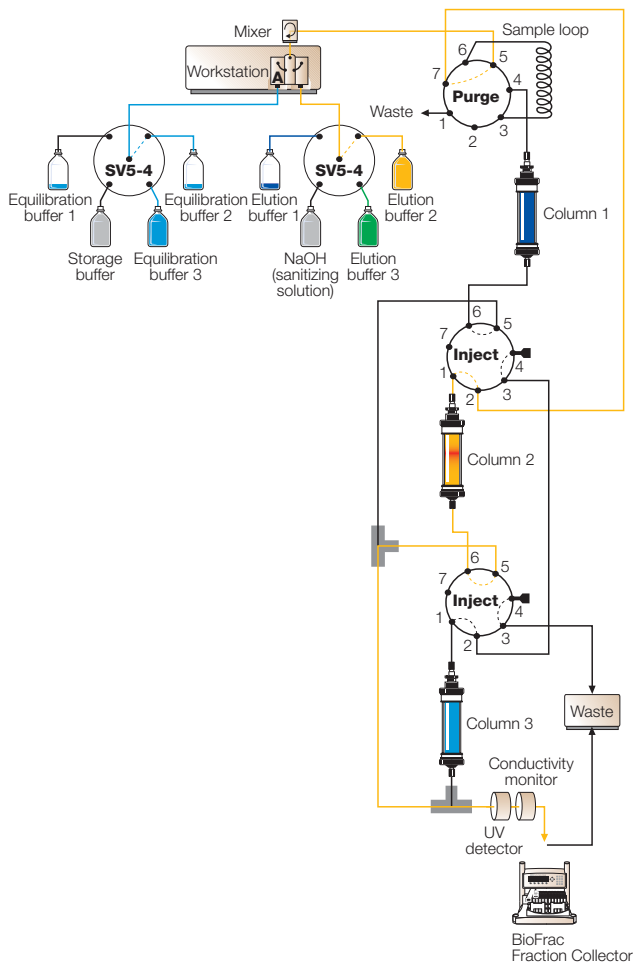


Step 4

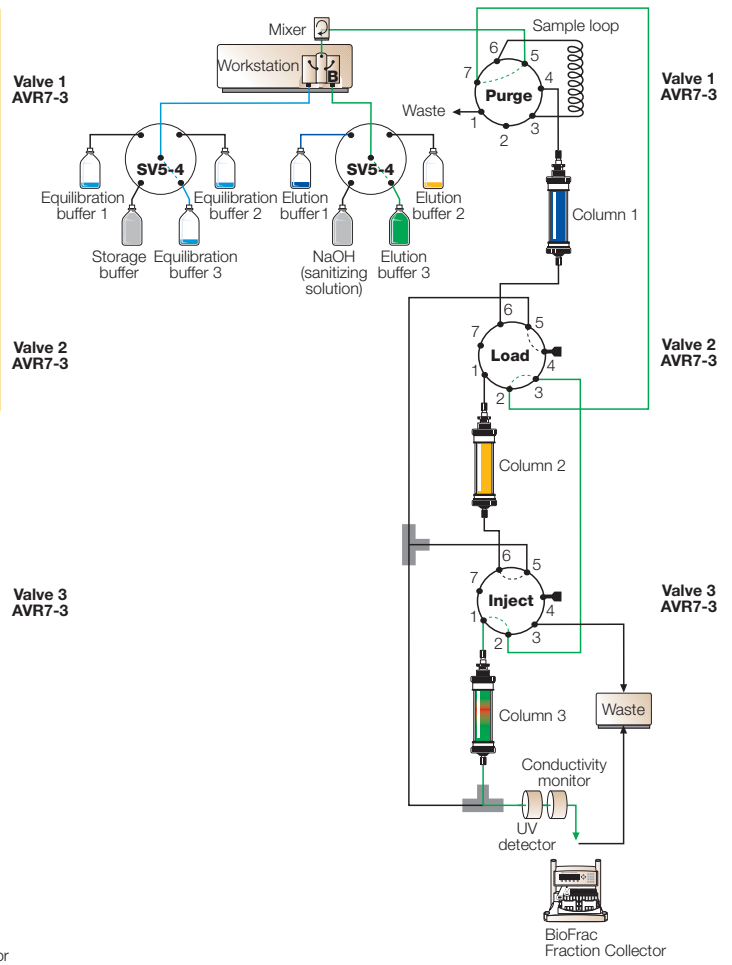
- To elute column 2, the gradient pump inlet valves both change to buffer 2
- Valve 1 changes to Purge position and valves 2 and 3 remain in Inject position
- The flow elutes column 2, bypassing columns 1 and 3, and goes to the fraction collector

Step 5

- To elute column 3, the gradient pump inlet valves change to buffer 3
- Valve 1 remains in Purge position, valve 2 is in Load position, and valve 3 remains in Inject position
- The flow elutes column 3 and goes to the fraction collector

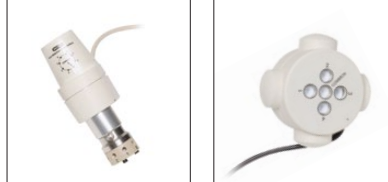


Step 4



Step 5

Application 12



Sequential Binding and Elution Using Three AVR7-3 Valves

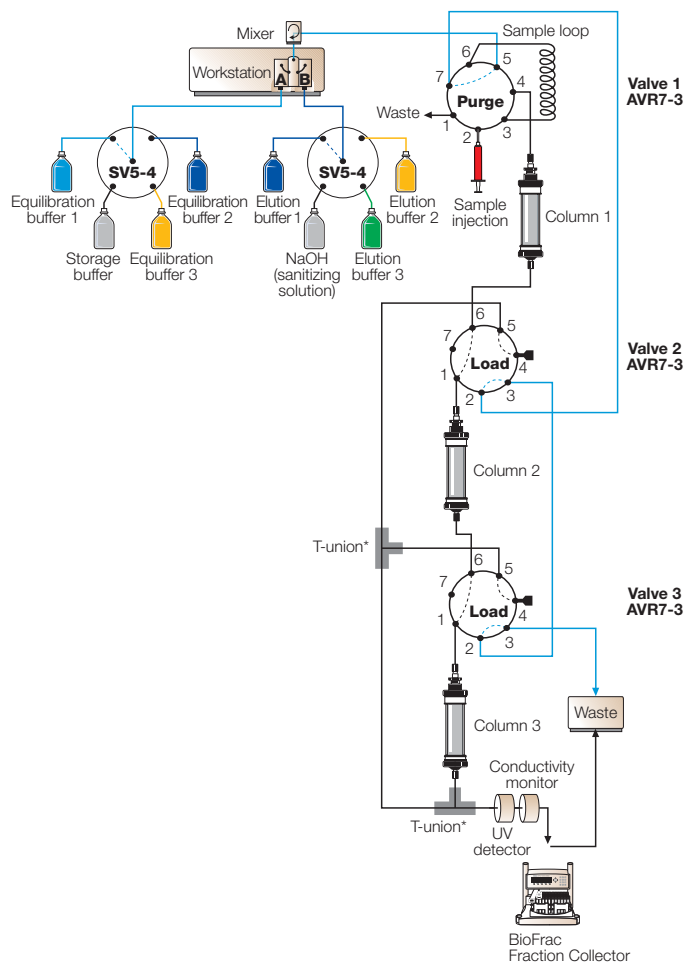
A sample is loaded onto one column, nonbinding proteins are washed away, and then a fraction of eluted proteins is diverted to a second column. This column is washed, eluted, and fractions diverted to a third column, which in turn is washed and eluted. Gradient pump inlet valves are used if different buffers are needed for equilibration and elution of each column.

Step 1

With valve 1 in Purge position and valves 2 and 3 in Load position, the gradient pump may be primed with the buffers from the gradient pump inlet valves. Two T-unions* are used to connect tubing. Port 4 of valves 2 and 3 should be end-capped to prevent misdirected flow.

To equilibrate columns individually:

Column	Valve 1	Valve 2	Valve 3
1	Load	Inject	Load or Inject
2	Purge	Inject	Inject
3	Purge	Load	Inject



Step 1

* P-12 TEE, with P-200/P-235, PEEK, available from Upchurch Scientific.

Application 12 (continued)



Step 2A (Not Shown)

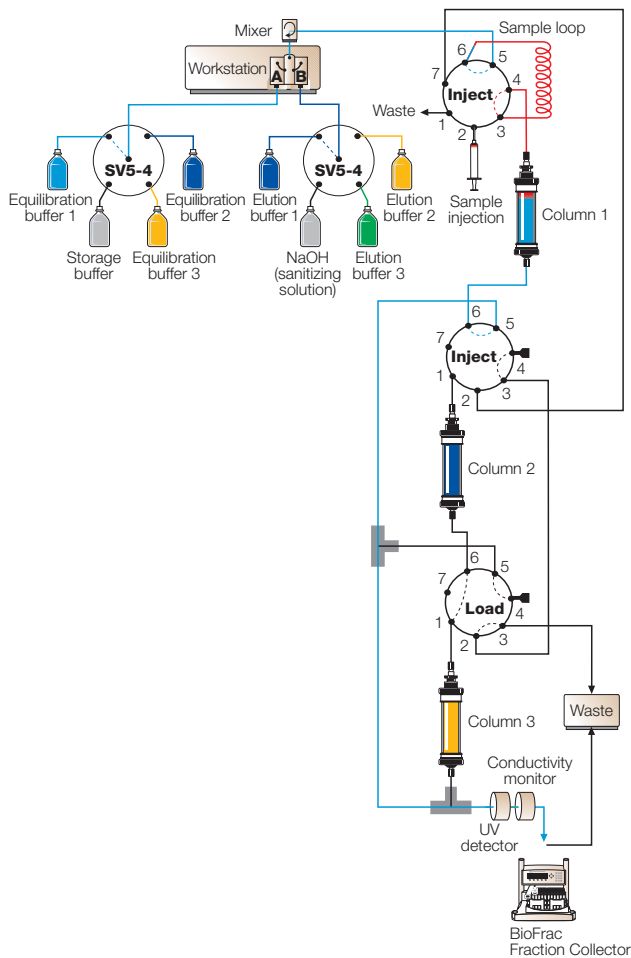
Valve 1 is placed in Load position to load sample into the loop. Valve 2 is in Inject position and valve 3 is in Load position. Columns 2 and 3 are now bypassed.

Step 2B

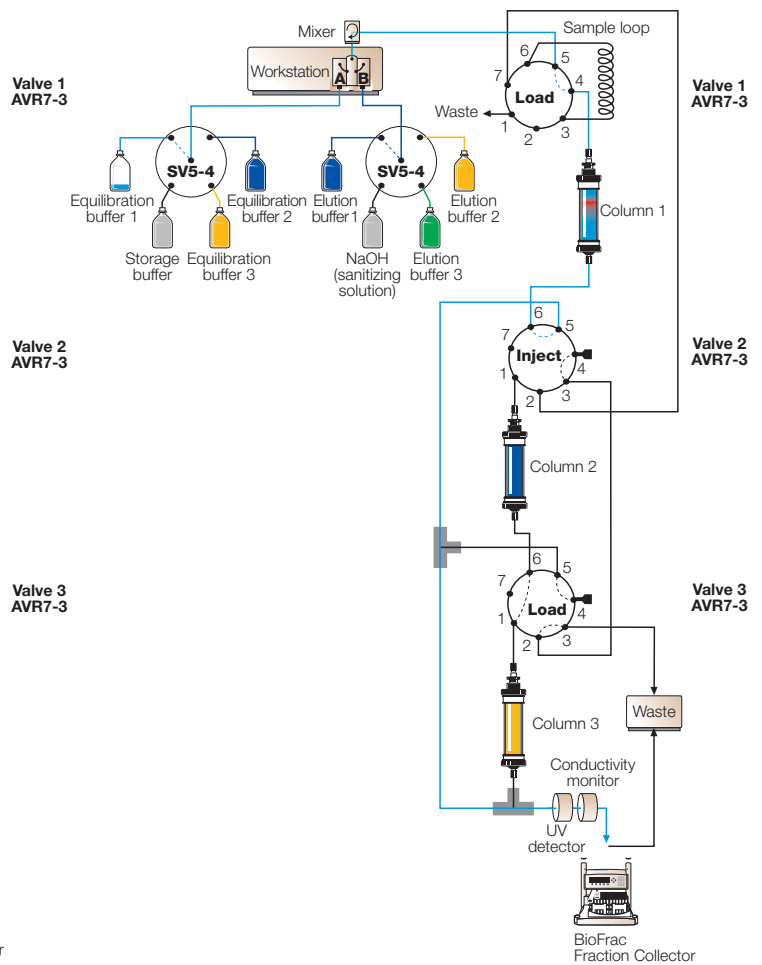
Valve 1 changes to Inject position and sample is loaded onto column 1.

Step 3

Valve 1 changes to Load position to wash column 1. The flow goes to the fraction collector or to waste via the fraction collector diverter valve.



Step 2B



Step 3

Application 12 (continued)

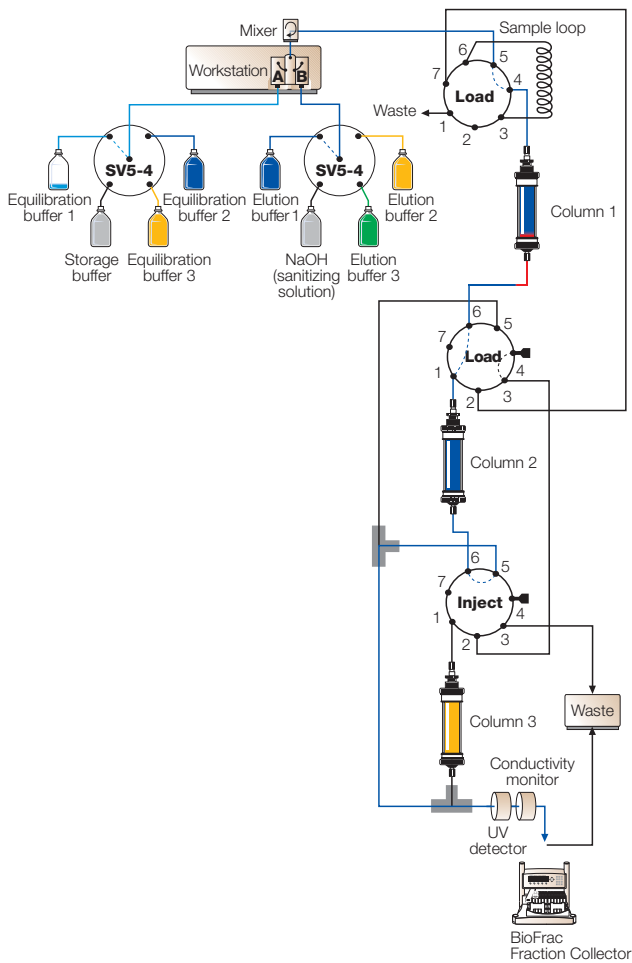


Step 4

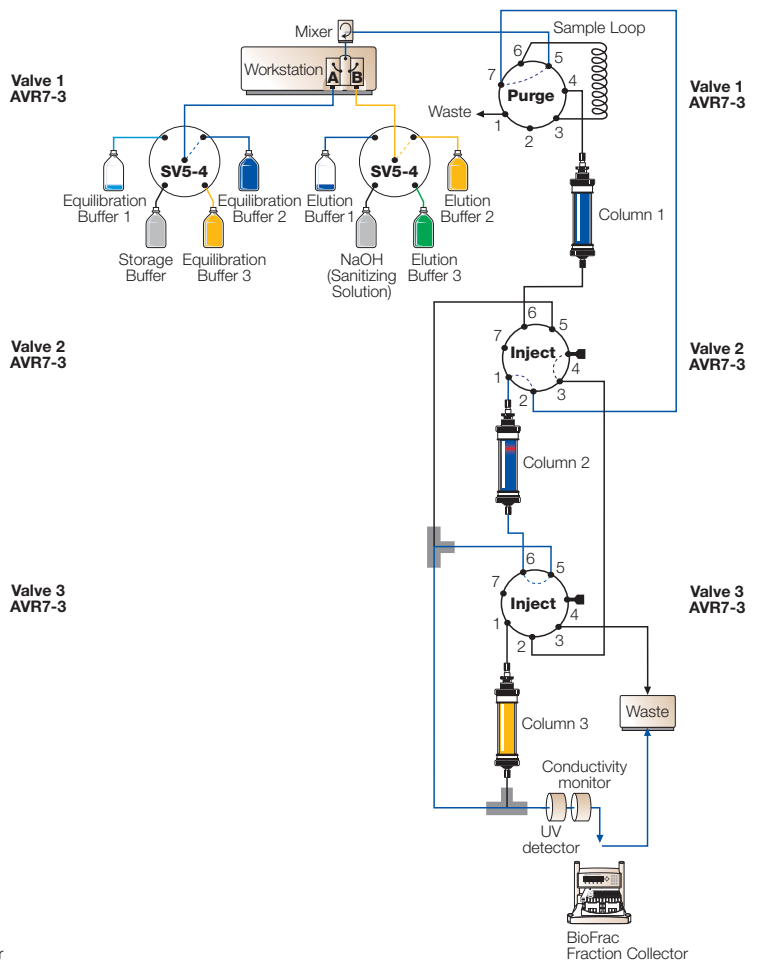
Valve 2 changes to Load position. The flow from column 1 passes through column 2 and then to the fraction collector. The flow path is shown.

Step 5

- Valve 1 changes to Purge position to bypass column 1
- With valves 2 and 3 in Inject position, the flow passes only through column 2 and then to the fraction collector (or waste via the fraction collector, as shown)



Step 4



Step 5

Application 12 (continued)

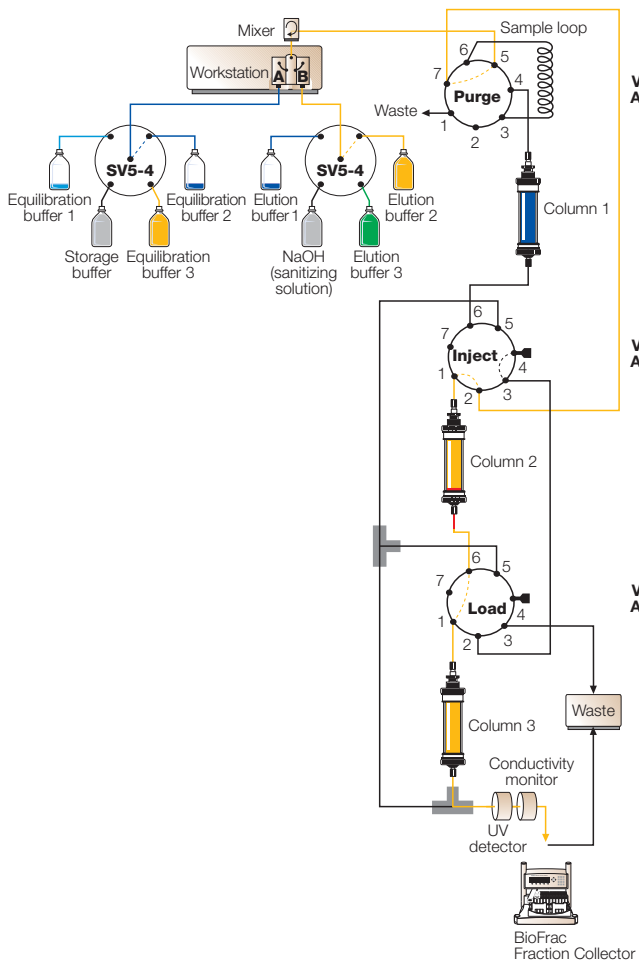


Step 6

- Valve 3 changes to Load position
- The flow passes through column 2 and column 3. The flow path is shown

Step 7

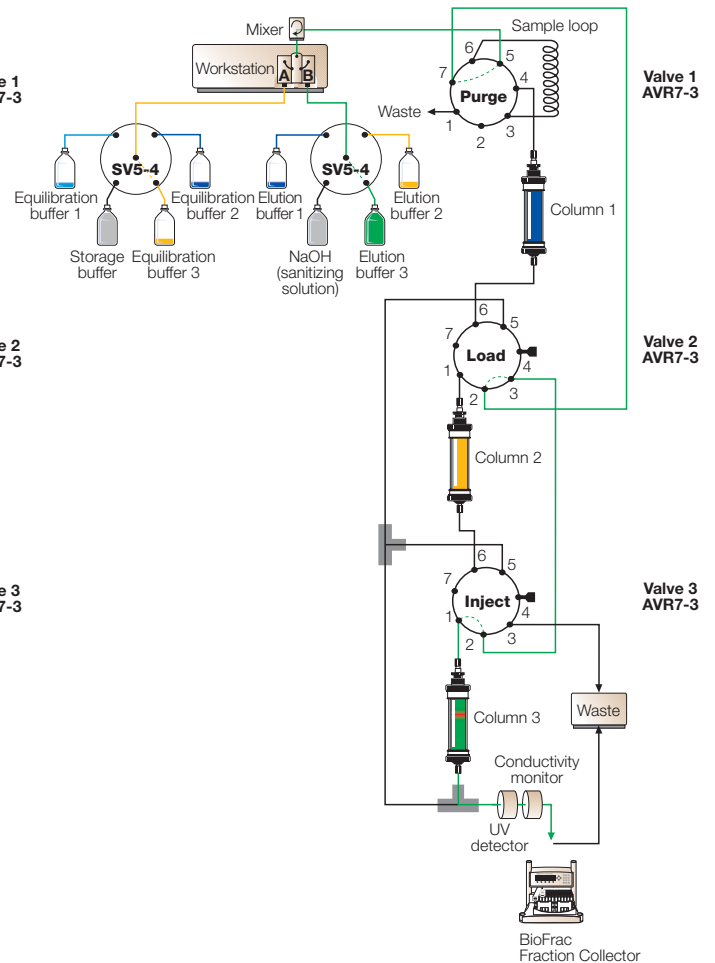
- Valve 2 changes to Load position. Column 2 is now bypassed
- Valve 3 changes to Inject position and column 3 is now washed and eluted (shown)



Valve 1
AVR7-3

Valve 2
AVR7-3

Valve 3
AVR7-3

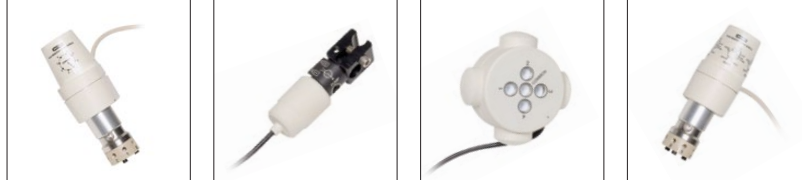


Valve 1
AVR7-3

Valve 2
AVR7-3

Valve 3
AVR7-3

Ordering Information



Catalog #	Description
BioLogic DuoFlow Valves	
760-0406	AVR7-3 Automated Sample Inject Valve , 7-port, 3-position high-pressure valve, 3,500 psi (233 bar) limit
760-0401	AVR7-3 Valve Rebuild Kit
760-0408	AVR9-8 Stream-Select Valve , 9-port, 8-position high-pressure valve, 3,500 psi (233 bar) limit
760-0403	AVR9-8 Valve Rebuild Kit
760-0410	SVT3-2 Diverter Valve , 3-port, 2-position solenoid valve, 30 psi (2 bar) limit
760-0411	SVT3-2 Valve Rebuild Kit
750-0415	SV5-4 Select Valve , 5-port, 4-position solenoid valve, 30 psi (2 bar) limit
BioLogic Dynamic (Sliding Piston) Loops	
750-0451	DynaLoop 25 Kit , includes 25 ml DynaLoop Sliding Piston Loop, DynaLoop Parts Kit, instructions
750-0452	DynaLoop 90 Kit , includes 90 ml DynaLoop Sliding Piston Loop, DynaLoop Parts Kit, instructions
750-0450	DynaLoop Parts Kit , includes 4 end cap O-rings, 1 sliding seal O-ring, 1 filter, 4 nut fittings, four 1/8" ferrules, five 1/4-28 nuts and ferrules, 10' of 1/8" PTFE tubing
750-0475	DynaLoop 25 Sample Loop , 25 ml, replacement
750-0476	DynaLoop 90 Sample Loop , 90 ml, replacement
750-0455	DynaLoop Sliding Seal Replacement
Model EP-1 Econo Pump	
731-8267	System Cable 7 , 8-pin mini-DIN to bare wires

Catalog #	Description
Econo Gradient Pump	
731-9001	Econo Gradient Pump , 100/120 V, includes tubing and fittings kits
731-9002	Econo Gradient Pump , 220/240 V
750-0651	System Cable 18 , bus communication cable, 3.7 m (12')
BioLogic Maximizer Valve System (for Use With a BioLogic DuoFlow System)	
760-2200	BioLogic Maximizer Kit , 100/120 V, for US, Japan, Korea; includes BioLogic Maximizer Base Unit, pH monitor, BioLogic Maximizer Mixer, starter kit, tubing kit, system cable 30, U.S. power cord

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Singapore 65 6415 3188 **South Africa** 27 (0) 861 246 723 **Spain** 34 91 590 5200 **Sweden** 08 555 12700 **Switzerland** 026 674 55 05
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