

Created:3/21/2024 11:27 AMFluidic Scheme:Default

Last Modified:5/14/2024 10:50 AMPump Head:F10

Project:ScottControl Flow Rate:No

Technique:Size ExclusionpH Monitoring:No

Method Notes: METHOD DESCRIPTION: This method is used to separate molecules on the basis of molecular size. Sample is applied after column equilibration, and eluted isocratically. Protein molecules separate and elute from the column according to their size (largest first).

COLUMN/SAMPLE: This method is designed for a 23-ml gel filtration column (but can be scaled to work with columns of different sizes. Typically, sample volumes should be 1-3 % of the column volume. The flow rate and max pre-column pressure limit vary depending on the selected column type.

MINIMUM REQUIRED DEVICES: Gradient Pumps A and B, Mixer module, Sample Inject Valve module, Single-Wavelength UV-Conductivity detector, BioFrac Fraction Collector

METHOD PARAMETERS
Flow Rate 1.0 ml/min
Sample Size: 2.0 ml
Fraction Size 1.0 ml
Fraction Collector Rack: F1 (12-13 mm x 100 mm tubes)

Fraction Collection:

Phase	Rack Type / Outlet Valve	Max Fraction Size (ml)
3	F1	0.50

Buffer Selection

Inlet A: Buffer A 1
Inlet B: Buffer B

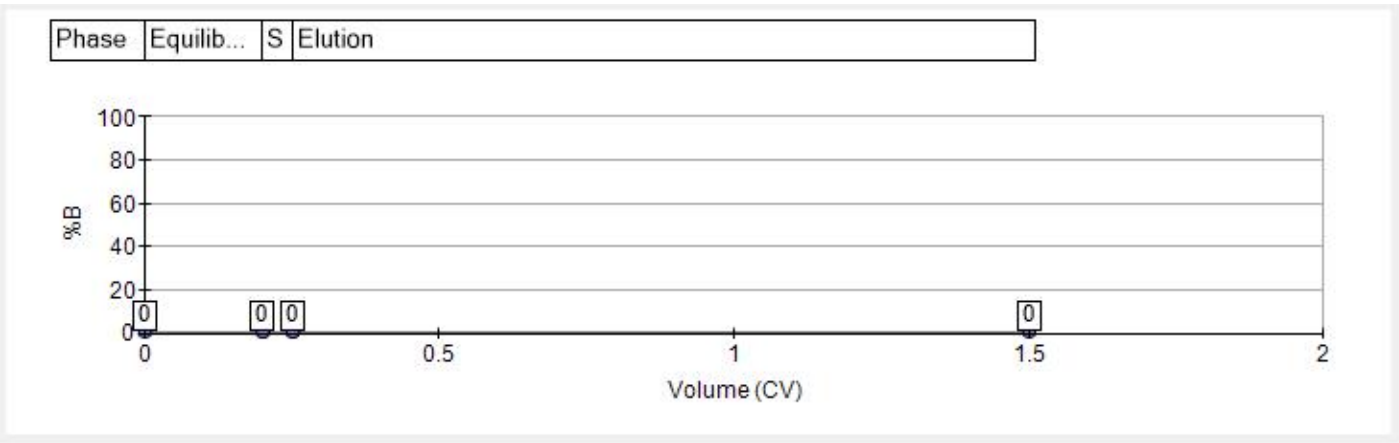
Columns

Position	Name	Volume	Max Pre Column Pressure	Max Delta Column Pressure	Default Flow Rate	Max Flow Rate
N/A	Superdex 75 10/300 GL	23.56 ml	200 psi	200 psi	0.5 ml/min	1 ml/min

UV Detector

Wavelengths: 214 nm, 260 nm, 280 nm

Gradient Graph



Method Steps

Step #	Total Vol (CV)	Step Description	Vol (CV)	Flow Rate (ml/min)	%B	Phase	Step Parameters
1	0	Gradient Segments	0.2	0	0	Equilibration	Forward Flow
1.1	0.2	Isocratic Flow	0.2	0.3	0	Equilibration	Buffer A 1, Buffer B
2	0.2	Hold Until (Disabled)	0	0	0	Equilibration	
3	0.2	Zero Baseline	0	0	0	Equilibration	
4	0.2	Fraction Collection (Waste)	0	0	0	Sample Application	
5	0.2	Load Inject Sample	0.05	0		Sample Application	
5.1	0.25	Inject Sample	0.05	0.3	0	Sample Application	System Pump Inject Loop, Buffer A 1, Buffer B
5.2	0.25	Change Valve (Sample Inject Valve)	0	0		Sample Application	Manual Load Loop / System Pump to Column
6	0.25	Fraction Collection (Frac. Size: 0.50 ml)	0	0	0	Elution	Scheme: Collect All (BioFrac)
7	0.25	Gradient Segments	1.25	0	0	Elution	Forward Flow
7.1	1.5	Isocratic Flow	1.25	0.3	0	Elution	Buffer A 1, Buffer B