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ProSwift C4 RP-5H Capillary Monolith

QuickStart

1. Overview

The ProSwift C4 RP-5H monolith capillary columns offer superior separation of biomolecules ranging from low to high molecular weight proteins including monoclonal antibodies. Conditioning of the column bed is **required** prior to initial use and after long-term storage. This QuickStart is intended to help first-time users quickly get started and also ensure extended column lifetime and reproducibility.

2. Preparation

A. Eluent Preparation:
The following eluents are recommended, but the column may be used with any eluent appropriate for analysis. Typically, Eluent A is a mobile phase with low organic content, and Eluent B is a mobile phase with high organic content.

- **Eluent A**: 0.1% TFA in water (Typical 95:5 v/v Water:CH_3CN)
- **Eluent B**: 0.1% TFA in CH_3CN (Typical 95:5 v/v CH_3CN:Water)

B. Column Installation:
Install the column on the instrument in the correct flow direction.

WARNING

Sudden increases in flow rates may damage monolithic columns. Always increase the flow rate slowly using a linear flow gradient or stepwise increments in flow rate.
If the eluent composition generates back pressure in excess of the maximum operating pressure, reduce the flow rate to ensure the upstream back pressure is less than the maximum operating pressure.

3. Flow Rate Start-Up

Using a linear or stepwise flow gradient, increase the flow rate of **Eluent B** starting from 0.00 µL/min to the desired flow rate over 5 minutes.

Part # /Parameter	Column Dimension	Flow Rate	Typical Pressure	Temperature
164935	50 µm x 25 cm	0.5 µL/min	< 1650 psi (11.4 MPa)	35°C
164928	100 µm x 50 cm	2.0 µL/min	< 3300 psi (22.8 MPa)	
164929	100 µm x 25 cm	2.0 µL/min	< 1650 psi (11.4 MPa)	
164930	200 µm x 25 cm	8.0 µL/min		
164931	500 µm x 10 cm	50 µL/min	< 660 psi (4.6 MPa)	
164932	500 µm x 25 cm	50 µL/min	< 1650 psi (11.4 MPa)	

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4. Column Conditioning

Use the guidelines below to determine the proper startup conditions:

A. Removal of Storage Solution:
   a. Using the desired flow rate, run a binary gradient from 100% B to starting conditions so that 10 column volumes pass through the column during the gradient. The table below indicates the total volume necessary for each column format
   b. Pump another 10 column volumes of starting eluent through the column.

Use the table below to determine the amount of eluent (10 column volumes) to flush through the column for the removal of storage solution in the above steps. Multiply the length of the column in cm by the volume factor to determine the 10 column volume in µL.
For example, for a 200 µm x 25 cm column, 10 column volumes = 25 cm X 1.88 = 47 µL.

Column Diameter	Volume Factor	Columnn Length (cm)	10 Column Volumes (µL)
50 µm	0.118	25	3.0
100 µm	0.471	25 / 50	11.8 / 23.6
200 µm	1.88	25	47.0
500 µm	11.8	10 / 25	118.0 / 295.0

B. Column Equilibration:
   a. Run the column at the desired starting eluent composition for 3 minutes.
   b. Then run a binary gradient to 100% B over 1 minute.
   c. Hold at 100% B for 5 minutes.
   d. Next run a binary gradient to your desired starting eluent composition over 1 minute.
   e. Repeat a – d until a reproducible background signal is obtained.

5. Storage

A. Store the column in 100% CH_3CN.

For additional information, please refer to the manual, PepSwift and ProSwift Capillary Monolith columns for Bioseparations, Product Manual Doc. No. 065592.

Part # /Parameter	Column Dimension	Recommended Flow Rate Range, µL/min	Maximum Pressure, psi/Mpa	Dynamic Binding Capacity per mL of Monolith
164935	50 µm x 25 cm	0.125 – 0.75	4950 (34.1)	40.0 µg/mL α-Chymotrypsinogen
164928	100 µm x 50 cm	0.5-3.0		
164929	100 µm x 25 cm	0.5-3.0		
164930	200 µm x 25 cm	2.0-12.0		
164931	500 µm x 10 cm	12-100	2000 (13.8)	
164932	500 µm x 25 cm	12-100		

Additional specifications for all formats; Max Temperature: 90°C; pH Limits: 2-10