

ProSwift[®] SAX-1S, WAX-1S, SCX-1S, and WCX-1S QUICKSTART

ProSwift Monolith columns offer superior separation of large biomolecules. Conditioning of the column bed is <u>required</u> prior to initial use and after long-term storage. The QuickStart process will ensure extended column lifetime and reproducibility.

I. Preparation:

a. Eluent Preparation

The following eluents are recommended, but the column may be used with any eluents suitable for analysis. Typically Eluent A is a low salt buffer and Eluent B is a high salt buffer.

Cation Exchange

0.01M Na₂PO₄ buffer. pH 7.6

Removal of Storage Solution:

to 100% B.

Column Equilibration:

conditions.

Using the desired flow rate, run a 0.4 mL (~10

column volume) binary gradient from 100% A

Pump another 0.4 mL (~10 column volumes)

Equilibration from 100% B to the starting

minute reverse gradient to the initial

eluent composition should include at least a 1

Pump at least 0.4 mL (~10 column volumes) of

this eluent composition through the column.

100% B through the column.

1 M NaCl in 0.01 M Na₂PO₄ buffer, pH 7.6

- Eluent Anion Exchange
- A 0.01 M Tris·HCl buffer pH 7.6
- *B* 1 M NaCl in 0.01 M Tris •HCl buffer pH 7.6
- b. Column Installation

Install the column on the instrument in the correct flow direction.

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 pressure, reduce the flow rate to ensure the upstream back pressure is less than the maximum pressure.

The maximum pressure limit for 4.6mm is 1000 psi (6.9 MPa) and for 1mm is 2000 psi (13.8 MPa).

II. Flow Rate Start-Up (Ramping)

Using a linear or stepwise flow gradient, increase the flow rate of Eluent A starting from 0.00 mL/min to the desired flow rate using the flow rates given below.

1 mm[.]

- a. 4.6mm Column: Use a rate of ≤ 0.50 mL/min, every 30 seconds
- b. 1mm Column: Use a rate of ≤ 0.10 mL/min every minute.
- III. Column Conditioning Use the guidelines below to determine the proper startup conditions:

4.6 mm:

WARNING

Removal of Storage Solution:

- Using the desired flow rate, run a 8 mL (~10 column volume) binary gradient from 100% A to 100% B.
- Pump another 8 mL (~10 column volumes) of 100% B through the column.

Column Equilibration:

- Equilibration from 100% B to the starting eluent composition should include at least a 1 minute reverse gradient to the initial conditions.
- Pump at least 8 mL (~10 column volumes) of this eluent composition through the column.

IV. Storage:

- a. For short-term storage, <3 days, store the column in the initial buffer compositions.
- b. For long-term storage, >3 days, use the following solution to avoid microbial growth on the column.

Anion Exchange

Cation Exchange

0.05 M NaCl in 0.01 M Tris•HCl, pH 7.6 + 0.1% NaN₃ 0.05 M NaCl in 0.01 M Na₂PO₄, pH 7.6 + 0.1% NaN₃

c. In all cases the column should be tightly sealed with end plugs to prevent the column from drying out.

For additional information, please refer to the manual, ProSwift Ion Exchange Product Manual Doc. No. 065122; see Section "System Requirements" regarding the use of stainless steel HPLC systems.

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