ProPac 3R quick start guide

Getting started

Prior to using Thermo Scientific™ ProPac™ 3R SCX and SAX 3 µm columns, review all the information in this section on column operation. Following these specifications for your column will help to ensure the column performs as it is intended and maximize the lifetime of your column. For more detailed information on column use please reference the ProPac 3R SCX and ProPac 3R SAX columns manuals online.

Column use and physical specifications

To ensure that you do not damage the column hardware or packed bed, take care to operate within the limits of the column. The table below indicates the operational limits for each column format in terms of flow rate, maximum column pressure drop from inlet to outlet, temperature, and mobile phase pH.

Column (PEEK)	Flow rate (mL/min)		Max column pressure drop[1] psi (bar)	Temperature °C	pH
	ProPac 3R SCX	ProPac 3R SAX			
4 × 100 mm	0.3-0.5	0.3-0.5	4500[2] (310)[2]	Ambient – 60°C	2-12
4 × 50 mm					
2 × 100 mm	0.1-0.2	0.1-0.2			
2 × 50 mm	0.1-0.3				

[1] The column pressure drop for a given flow rate is calculated as the pressure of the system with column minus the pressure of system with union in place of column.
[2] For PEEK body columns, the maximum pressure at the column inlet should not exceed 7000 psi (485 bar) to avoid damaging the column body.

Additional requirements for safe column operation:

- Always set up the mobile phase flow direction as indicated on the column tag
- Avoid exposing the column bed to sharp pressure fluctuations that may disrupt the column bed
- When starting, stopping, or changing the flow rate, a flow ramp rate (mL/min/min) of ~1/3 of the maximum flow rate for the specific column format is recommended

Recommended buffers for salt and pH gradient separations

Salt gradient separations typically offer the best resolution possible for individual applications. Please consult the table below for recommended buffer conditions to achieve optimal separations and maintain good column performance throughout its lifetime.

Parameter	Recommended	
	ProPac 3R SCX	ProPac 3R SAX
Buffer	• MES or other Good's buffers • Thermo Scientific™ CX-1 pH gradient buffer • LC-MS: ammonium acetate, ammonium bicarbonate, ammonium formate and associated acids and bases[4]	• Tris or other Good's buffers • LC-MS and pH gradient buffer: ammonium acetate, ammonium bicarbonate, ammonium formate and associated acids and bases for pH gradients[4]
Minimum salt concentration	• 20 mM NaCl to avoid high pressure that can damage the column stationary phase • **CAUTION:** Never use pure deionized water on the column as this will result in irreversible damage	
Detergent additives	• Nonionic, anionic or zwitterionic detergents • **CAUTION:** Do not use cationic detergents as they will irreversibly bind to the column and reduce the separation power	• Nonionic, cationic or zwitterionic detergents • **CAUTION:** Do not use anionic detergents as they will irreversibly bind to the column and reduce the separation power
Organic solvent compatibility[3]	• Up to 20% acetonitrile • Up to 10% methanol	
Cleaning agents	• For metal contamination (Fe, Cu, etc.) removal, flush the column at 0.4× the max column flow rate for 12 hours with 10mM EDTA + 50mM NaCl adjusted to pH 8.0	
Storage solution	• Short term (<24 hrs): ≥ 20 mM NaCl and your application buffer • Long term (>24 hrs): ≥ 20 mM NaCl and your application buffer + 0.1% sodium azide	

[3] Acetonitrile and methanol have viscosity maxima when mixed with water at certain ratios. This may cause unexpectedly high pressure. Always use low flow rates until the pressure behavior is understood when using these chemicals. Mixtures of ACN and MeOH should be introduced and removed gradually from the column using a gradient over 20 minutes to ensure a sharp viscosity front does not result in a rapid pressure difference in-column that may damage the packed bed.
[4] Due to the weak ionic strength of volatile pH buffers, use lower flow rates for initial method development until the column back pressure is understood. The flow rate can then be increased as needed while still observing the maximum allowed pressure for the column.

Column conditioning

Your column has been designed to minimize secondary interactions and for low carryover. Depending on the nature of your sample, column conditioning may be required prior to achieving optimal performance. To quickly condition your column, we recommend performing 1-2 sample overload injections of 10× your standard sample loading and standard gradient method.

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