CARE AND USE MANUAL

Xtimate SEC HPLC Column Care and Use Manual

Welch

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Xtimate SEC HPLC Column
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Dear User:

Thank you for choosing our liquid chromatography column products! This manual is designed to help you use our products under optimal conditions and maximize column lifespan. Please read the usage instructions carefully before operation.

Xtimate SEC column employs high-purity, highly stable spherical silica packing and a proprietary chemical-bonding process that grafts hydrophilic polymers and diol functional groups onto the silica surface. The dual-bonding mechanism minimizes nonspecific adsorption of water-soluble polymers, proteins, biomolecules, enzymes, and peptides. It is widely used for the separation and analysis of water-soluble polymers and biological macromolecules.

Preparation Before Use

Column Identification

To ensure the quality of every column, each column sold by Welch Materials is assigned a unique identification code. This code allows us to trace quality-related issues. Upon receiving your column, please follow these steps:

1. Verify that the packaging is intact and matches the ordered column model.
2. Ensure the package contains a COA report with the inspector's signature.
3. Check the column surface for any damage and confirm that the protective plugs on both ends are intact.
4. Verify that the column body bears a Welch Materials identification label. Confirm that the model and serial number on the box match those on the column label.

Note:

The packing material inside the column is composed of fine particles. Do not attempt to open the column arbitrarily, as the material may become airborne and pose a health risk. If opening is necessary, use appropriate protective measures.

Installation

To achieve optimal column efficiency, use narrow-diameter tubing for connections. The connections to the column inlet and outlet must be tightly sealed, with the tubing ends polished and free of burrs or bevels. Use professional tools to cut and trim tubing.

1. Remove the column from its packaging. Check the direction of the eluent flow indicated by the arrow on the column body. Unscrew the protective plugs at both ends of the column. Position the column so that the eluent flow aligns with the direction indicated, as shown in Figure 1.

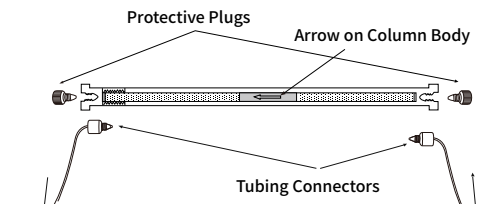


Figure 1. Column Installation

2. Carefully screw stainless steel or other compatible material fittings into both ends of the column. Ensure tight, zero-dead-volume connections to prevent leakage during operation.

Product Specifications

Column	Xtimate SEC
USP Code	L33/L59
pH Range	2.0–7.5, short term 7.5–9.5
Max. Temperature	80 °C
Max. Pressure Tolerance	4500 psi

Note:

When using silica-based columns at pH ＞7, use buffers with concentrations not exceeding 20 mM and temperatures not exceeding 40 °C to maximize column lifespan. Extreme pH or temperature conditions can significantly reduce column durability.

Product Specifications

Bonded Phase	Pore Size	Protein Molecular Weight Range	Water-soluble Macromolecule Range
Xtimate SEC-120	120 Å	500–150,000	500–25,000
Xtimate SEC-200	200 Å	500–200,000	500–50,000
Xtimate SEC-300	300 Å	5,000–1,250,000	1,000–100,000
Xtimate SEC-500	500 Å	10,000–3,500,000	2,000–500,000
Xtimate SEC-700	700 Å	15,000–5,000,000	2,500–500,000
Xtimate SEC-1000	1000 Å	50,000–7,500,000	5,000–1,500,000
Xtimate SEC-2000	2000 Å	>10,000,000	50,000–2,500,000

Column Activation

(Recommended for new columns or those unused for over a month)
Xtimate SEC columns are shipped in a solution of 90% acetonitrile in water. Activation should follow these steps:

Step 1: Activation

Activation Procedure		
Specification	ID≤4.6 mm	ID>4.6 mm
Flow Rate	0.2 mL/min	0.5 mL/min
Mobile Phase	100% Acetonitrile → 100% Water	
Time	5 hours each	
Temperature	30 °C	

Routine Column Washing

Method Mobile Phase	Without acids, bases, salts	Containing acids, bases, salts
Washing Mobile Phase	10% → 90% Acetonitrile	5% → 90% Acetonitrile
Flow Rate	Method Flow Rate	
Time	40 mins each (Column Length ⩽100mm)	
	60 mins each (Column Length >100mm)	
Storage	Store the column in 90% acetonitrile, and place in a cool, dry place.	

Column Regeneration:

After repeated use, certain sample components may adsorb to the inlet frit or packing material. When accumulation reaches a critical level, abnormal increases in backpressure may occur, accompanied by peak broadening, necessitating a targeted cleaning flush. Basic principles for selecting the cleaning solution are:

1. Low-pH saline solutions assist in removing basic proteins.
2. Organic solvents assist in removing hydrophobic proteins.
3. Solubilizing agents assist in removing strongly adsorbed species from the stationary phase (such as those bound via hydrogen bonding).

Note: Use solubilizing agents only when washes with neutral saline or organic solvents do not produce a clear improvement (e.g. 6–8 M urea or 0.2–0.3% sodium dodecyl sulfate).

Column Regeneration (continued):

Flow Rate	1/2 of Method Flow Rate
Mobile Phase	Step 1: Low pH, high concentration neutral salt solution (e.g 0.5M sodium sulfate，pH adjusted to 3.0 with sulfuric acid) Step 2: Buffer solution (e.g 50mM PB Solution, pH 7.0) containing organic solvents (e.g 10% to 20% methanol, acetonitrile, or ethanol)
Time	60 mins each (Column Length ⩽100mm)
	120 mins each (Column Length ＞100mm)

Glossary of Terms:

pH Range: The pH range tolerable for the column's mobile phase and sample solutions.

Specific Surface Area: The surface area per gram of silica.

Pore Size: The diameter of pores within silica particles, selected based on analyte molecular weight.

Carbon Loading: The percentage of carbon content in bonded functional groups relative to the total silica mass.

USP Code: Universal code for bonded phases in the USP chromatography column database.

Maximum Pressure Tolerance: The upper pressure limit the column can withstand. Long-term use is recommended at pressures below 30 MPa. Monitor the instrument's pressure for safe operation.

Storage Solvent: Solvent used during storage or transport to maintain the activity of packing material. Handle with appropriate protective measures.