CARE AND USE MANUAL

Xtimate Bio SEC HPLC Column Care and Use Manual

Welch

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Xtimate Bio SEC HPLC Column
Care and Use Manual

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 Bio SEC column features ultrapure silica microspheres as matrices, with a uniform surface bonded with a hydrophilic polymer of nanometer thickness. It effectively reduces the adsorption of biological samples on the column, making it ideal for the analysis of proteins, antibodies, cytokines, and other biological samples.

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.

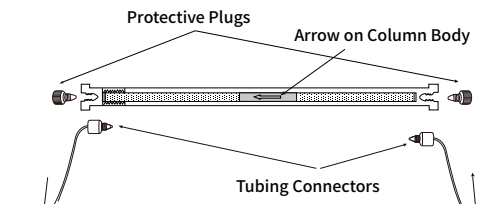


Figure 1. Column Installation

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 Bio SEC
USP Code	L33/L59
pH Range	2.0–8.0
Max. Temperature	40 °C
Max. Pressure Tolerance	100 bar (5 µm)
	200 bar (3 µm)

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
Xtimate Bio SEC-120	100 Å	100–100,000
Xtimate Bio SEC-120	120 Å	500–150,000
Xtimate Bio SEC-150	150 Å	500–200,000
Xtimate Bio SEC-200	200 Å	1,000–500,000
Xtimate Bio SEC-300	300 Å	5,000–1,250,000
Xtimate Bio SEC-500	500 Å	15,000–5,000,000
Xtimate Bio SEC-1000	1000 Å	50,000–7,500,000

Column Activation

(Recommended for new columns or those unused for over a month)
Xtimate Bio SEC columns are shipped in a solution of 20% ethanol. 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% Water	
Time	5 hours	
Temperature	30 °C	

Column Maintenance and Regeneration

• Ionic adsorption: Clean the column with a high-salt (e.g., 0.25 mol/L) wash solution or with acidic aqueous solutions such as phosphate buffer (pH 2.5).

• Hydrophobic adsorption: Add organic solvent (e.g., methanol or acetonitrile, ~15% v/v) to the wash solution and flush at a low flow rate. Prior to this organic wash, perform a transitional rinse with pure water to prevent salt precipitation from the buffer.

• Hydrogen-bonding adsorption: Prepare a wash solution containing 6 mol/L urea or 0.2% neutral surfactant (e.g., Triton, Tween, Brij, etc.), then thoroughly rinse the column with pure water to remove residual urea and surfactant.

• Frequent solvent exchanges can shorten column lifetime; therefore, only regenerate the column when necessary. Typical wash volumes are 5–10 column volumes. During cleaning, keep column pressure, flow rate, and wash solution pH within the recommended operating ranges to avoid damage.

Note: Do not attempt to open the column arbitrarily.

Column Storage

When not in use, ensure both ends of the column are securely capped to prevent solvent evaporation and salt precipitation.

Short-term storage (within 5 days): Keep the capped column in a low-salt, neutral pH buffer (e.g., 50 mmol/L phosphate buffer, pH 7.0).

Long-term storage: For extended idle periods, to prevent microbial growth or corrosion of stainless-steel housings (e.g. from chloride ions), store the column in 10–20% ethanol (or methanol or acetonitrile) in water, or in buffer containing 0.05% sodium azide. If necessary, refrigerated storage at 5–10 °C is acceptable.

Note: Frequent replacement of the column solvent will reduce column service life.

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.