Technical
User Guide

Evolve®D Process Columns

Product Codes:

AD070050	AD070100	AD070150	AD070200
AD100050	AD100100	AD100150	AD100200
AD140050	AD140100	AD140150	AD140200
AD200050	AD200100	AD200150	AD200200

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PURITY by DESIGN

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1. INTRODUCTION

Evolve®D

The Evolve®D Column range is a disposable 'single-use' format column designed specifically for use in Biopharmaceutical applications.

These columns are supplied pre-packed and ready to use. The entire flow path is non-metallic, which has the following advantages:

- Eliminates the corrosion risk and contamination of the process
- Allows the use of high concentration sodium chloride solutions and guanidine hydrochloride
- Prevents any product inactivation through metal contact

The columns are designed to be an open platform to support Astrea Bioseparations products as well as a wide range of customer chromatography media. The columns are qualified in terms of performance and pressure flow characteristics.

Quality Standards

The columns are available as a Process-Ready or a non-Process-Ready product. The non-Process-Ready columns are packed in a non-controlled environment but utilize the same quality columns and packing procedures as the Process-Ready columns. The Process-Ready columns are packed with all appropriate associated documentation and controls in a classified ISO Class 7 environment, and suitably sanitised to allow direct implementation into processes without the need for pre-preparation by the user. All materials in contact with the process stream have been selected for their suitability for use in equipment utilised in biopharmaceutical applications, and so either conform to the relevant sections of FDA code of Federal Regulations vol. 21 170-199 and/or passed USP class VI testing for in vivo toxicity.

2. Safety Guidelines

Safety Considerations

- Please read through this user guide prior to use to ensure safe and careful handling of the product.
- Please review the Regulatory Support File for additional information regarding the product.
- The Evolve®D pre-packed columns may be shipped in an ethanol-based preservative. This solution is required to be flushed from the product during equilibration and preparation for use and disposed of appropriately.
- Please follow all local laws and regulations for safe disposal of the column
- For laboratory (non-Process-Ready) and manufacturing production (Process-Ready) use only and is not for diagnostic purposes or for administration into humans.
- Please note that the non-Process-Ready Evolve®D columns are prepared and packed in a non-certified environment. The Process-Ready Evolve®D columns are prepared and packed in an ISO7 certified environment. All packed Evolve®D columns are classified as non-sterile.

Pressure Equipment Directive (PED)

European Pressure Directive 97/23/EC relates to regulations that are mandatory and govern a wide range of pressure containing vessels. The design and manufacture of chromatography columns, including the Evolve®D columns, are affected by these regulations.

The regulations are divided into categories, and the Evolve®D column range has been assessed and found that it falls within the Sound Engineering Practice (SEP) category. The foundation of the assessment is based on the following:

- The range of Evolve®D columns are liquid chromatography columns and when used in accordance with their Operating Instructions there should be no gas e.g. air present in the columns.
- The substances that will be used in the column will remain in liquid form during the entire operation at the maximum stated operating temperature.

In addition:

- The maximum pressure is less than 6 bar.
- The product pressure x volume is equal to or less than 200 bar litres.

Handling

Care should be taken when handling the columns; dependent upon size, they can weigh up to 17 kg (for the pre-packed AD200200) and so additional safe handling measures may be required. Mechanical handling equipment or manual handling aids should be utilised where personnel cannot safely lift or move the column or its subassemblies.

Chemical Compatibility

This user guide lists those substances for which the column hardware is compatible (see Section 4). If substances not listed are to be used the user must consider their effect as the integrity and safety of the column could be compromised.

3. Column Properties

Evolve®D 70 mm Columns Technical Data

Column	AD070050	AD070100	AD070150	AD070200
Volume	193 mL	385 mL	578 mL	770 mL
Bed height	5 cm	10 cm	15 cm	20 cm
Max. external diameter	18 cm	18 cm	18 cm	18 cm
Max. external height	23 cm	28 cm	33 cm	38 cm
Inner diameter	7 cm			
Cross sectional area	38.5 cm^2			
Maximum recommended operating pressure*	4 bar (58 psi)			
Operating temperature	2 – 30 °C			
Inlet/outlet diameter	3 mm			
Inlet/outlet connections	¾” (25 mm) sanitary tri-clamp			
Bed support	8 µm polypropylene mesh			

*See appropriate adsorbent technical user guide for specific information on operating conditions (pressure, buffers etc)

Evolve®D 100 mm Columns Technical Data

Column	AD100050	AD100100	AD100150	AD100200
Volume	393 mL	785 mL	1178 mL	1570 mL
Bed height	5 cm	10 cm	15 cm	20 cm
Max. external diameter	18 cm	18 cm	18 cm	18 cm
Max. external height	23 cm	28 cm	33 cm	38 cm
Inner diameter	10 cm			
Cross sectional area	78.5 cm^2			
Maximum recommended operating pressure*	4 bar (58 psi)			
Operating temperature	2 – 30 °C			
Inlet/outlet diameter	3 mm			
Inlet/outlet connections	¾” (25 mm) sanitary tri-clamp			
Bed support	8 µm polypropylene mesh			

*See appropriate adsorbent technical user guide for specific information on operating conditions (pressure, buffers etc)

Evolve®D 140 mm Columns Technical Data

Column	AD140050	AD140100	AD140150	AD140200
Volume	770 mL	1540 mL	2310 mL	3080 mL
Bed height	5 cm	10 cm	15 cm	20 cm
Max. external diameter	22 cm	22 cm	22 cm	22 cm
Max. external height	23 cm	28 cm	33 cm	38 cm
Inner diameter	14 cm			
Cross sectional area	153.9 cm^2			
Maximum recommended operating pressure*	4 bar (58 psi)			
Operating temperature	2 – 30 °C			
Inlet/outlet diameter	6 mm			
Inlet/outlet connections	¾" (25 mm) sanitary tri-clamp			
Bed support	8 µm polypropylene mesh			

*See appropriate adsorbent technical user guide for specific information on operating conditions (pressure, buffers etc)

Evolve®D 200 mm Columns Technical Data

Column	AD200050	AD200100	AD200150	AD200200
Volume	1571 mL	3142 mL	4713 mL	6284 mL
Bed height	5 cm	10 cm	15 cm	20 cm
Max. external diameter	34 cm	34 cm	34 cm	34 cm
Max. external height	25 cm	30 cm	35 cm	40 cm
Inner diameter	20 cm			
Cross sectional area	314.2 cm^2			
Maximum recommended operating pressure*	4 bar (58 psi)			
Operating temperature	2 – 30 °C			
Inlet/outlet diameter	6 mm			
Inlet/outlet connections	¾” (25 mm) sanitary tri-clamp			
Bed support	8 µm polypropylene mesh			

4. Column Hardware

Material Conformity

All materials in contact with the process stream have been selected for their suitability for use in equipment utilised in Biopharmaceutical applications and so either conform to relevant sections of FDA code of Federal Regulations vol. 21 170-199 and/or passed USP class VI testing for in vivo toxicity.

Materials of Construction – process wetted parts

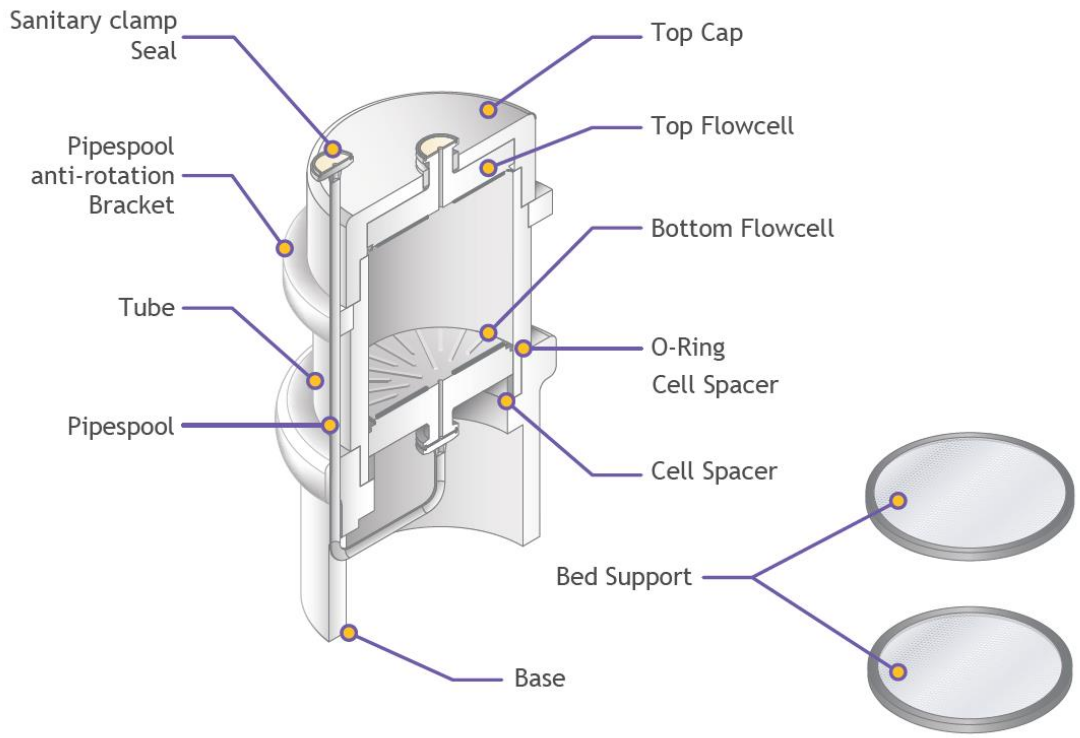
Column component	Material
Column tube	Polypropylene
Sanitary clamp blanking caps	20% Glass filled polypropylene
Sanitary clamp adaptors	20% Glass filled polypropylene
Bed supports (mesh) – 8 µm	Polypropylene
Adjuster (bottom) bed support integral seal	Santoprene®
Adjuster seal (O-ring)	EPDM
Fixed (top) bed support integral seal	Santoprene®
Fixed (top) flow cell	Polypropylene (white)
Adjuster (bottom) flow cell	Polypropylene (white)
Anti-jet	20% Glass-filled (GF) polypropylene
Sanitary clamp seals	Santoprene® elastomer
Pipe spool	Polypropylene

Materials of Construction – non-wetted parts

Column component	Material
Column top cap and base	Acetal, White
Pipe spool anti-rotation bracket/ Column Handle	Acetal, White
Cell spacer	Polypropylene
Bolts/lock pins (grub screws)	316 Stainless Steel
Clip clamp, ¾” ladish (Sanitary clamp)	Nylon & GF polypropylene, Black
Spacer	EPDM

Column Schematic

Figure 1: Evolve® D column design (cut through schematic)



Chemical Compatibility

Evolve®D columns are ideally suited for aqueous-based applications. See the table below for detailed compatibility information.

Note: The chemical compatibility of the packed material within the column may be different to those listed in the following tables. Please check/consider the chemical compatibility of the packed media with the resin/adsorbent supplier.

Resistance	++	Resistant; suitable for continuous use
	+	Limited resistance
	-	Not resistant; not recommended
	n/a	No information available
Notes	General	All solvents are at 100% concentration unless stated otherwise Concentrations: % refer to w/w All data is referenced to room temperature (15ºC to 25 ºC)

Chemical	Wetted Components	Non-Wetted
1,2-dichloroethane	-	++
Acetic acid 25%	++	++
Acetone 5%	++	++
Acetonitrile	-	++
Ammonia aqueous < 25%	++	++
Ammonium Sulphate 10-40%	++	++
Benzyl alcohol up to 2%	++	++
Butanol	+	++
Calcium chloride	++	+
Calcium hydroxide 30%	++	++
Calcium hypochlorite	++	+
Chloroacetic acid 50%	-	++
Chloroform	-	++
Chromic acid 10%	-	-
Citric acid	++	++
Copper sulphate	++	++
Dichloromethane	-	++
Dimethyl formamide	-	++
Dimethyl sulfoxide 10%	++	++
Disodium phosphate	++	++
Ethanol 20%	++	++
Ethanol 70%	-	++
Ethylene glycol (1,2-ethanediol)	++	++
Formaldehyde 50%	++	++
Glycerol (Glycerine)	++	++
Guanidine hydrochloride 6M	++	-
Hydrochloric acid 10%	++	-
Hydrofluoric acid	++	-
Hydrogen peroxide	+	++
Industrial methylated spirit (IMS)	-	++

Chemical	Wetted Components	Non-Wetted
Methanol 20%	++	++
Methanol 50%	-	++
Methyl chloride (Chloromethane)	-	++
Methyl ethyl ketone (MEK)	-	++
Methylene chloride	-	++
Nitric acid 10%	++	++
Nitric acid 70%	-	+
Peracetic acid 300 ppm	++	++
Phosphoric acid < 55%	++	++
Potassium hydroxide 2 M	++	++
Iso-propanol 20%	++	++
Iso-propanol 40%	-	++
n-propanol 20%	+	++
Sodium acetate	++	++
Sodium bicarbonate 20%	++	++
Sodium carbonate	-	++
Sodium chlorate	++	++
Sodium chloride 2 M	++	++
Sodium chloride 6 M	++	-
Sodium hydroxide up to 2 M	++	++
Sodium hypochlorite 200 ppm	++	-
Sodium nitrate	++	++
Sodium sulphate	++	-
Trichloroethene (Trichloroethylene)	-	-
Triton® X-100 surfactant	++	++
Urea 6 M	++	++
Zinc chloride	++	++

5. Column Installation and Operation

Column Installation

The column is designed as a disposable, ready to use unit; the following steps provide guidelines to assist the installation of the column.

1. Remove the column from the external packaging (cardboard box).

2. The column will either be in a single bag (non-Process-Ready) or double bagged (Process-Ready). Remove the plastic bagging.

3. Place the column on a level surface and remove the black PVC click clamp covers.

4. Remove the sanitary caps by unclipping the sanitary clamps on both the inlet and outlet. The sanitary clamps are re-useable and are removed by pushing one of the ratchet end perpendicular from the other ratchet end (please note that the ratchet ends are one directional). The inlet port is the central port in the middle of the column and the outlet port is attached to the side of the column by means of a pipe spool.

5. Connect the inlet of the column to a chromatography system which has been cleaned, primed and set at a slow flow rate to minimize any risk of air entering the column with 25 mm tri-clamp (TC) sanitary fittings. A 4-port 2-way valve can also be used to create this connection. The Santoprene® gaskets can be re-used to form a seal with the 25 mm TC sanitary fitting from the chromatography system. Fit a tri-clamp or sanitary clamp around the two sanitary fitting flanges and secure firmly (if re-using the click clamps, press the two ratchet ends together firmly).

6. Connect the outlet of the column to the chromatography system as described in step 5.

7. The column is transported containing a storage solution/preservative (ethanol-based solution or other; please consult the relevant user guide of the packed adsorbent/resin). This will need to be flushed out prior to use. The recommended flow rate is 50% of operational flow rate (e.g. if the operational flow rate is 120 cm/h, set the initial flush flow rate at 60 cm/h) using an appropriate equilibration buffer. Allow at least three column volumes of the buffer to flow through, until the eluent matches the pH and conductivity of the buffer/solution entering the column. The column may be further conditioned prior to use by passing at least three column volumes of running buffer through the column in reverse flow, followed by a further three column volumes in down-flow.

8. If there is a requirement to evaluate the column efficiency, please following the instructions as described in Appendix 1.

Column Operation

The following recommendations are not prescriptive; please refer to the individual user guide of the packed media with respects to flow rates, pressure recommendations, and other process conditions. Thorough investigation of these parameters at small-scale is recommended to reveal the level of flexibility that can be tolerated with the chromatography media, buffer and protein combination selected.

- Filter all buffers and feedstock through an appropriate filter prior to running the column, 0.22 µm is recommended.
- The maximum operating pressure for the Evolve® D column is 4 bar - do NOT exceed this pressure limit, regardless of whether the packed media can withstand higher.
- As previously mentioned, the column inlet is located on the top and in the centre of the column, and the column outlet is located on the top of the column to the side via a pipe spool from the bottom of the column.
    - However, if required (e.g. for CIP purposes), the column may be operated in reverse flow

	AD070050 AD070100 AD070150 AD070200	AD100050 AD100100 AD100150 AD100200	AD140050 AD140100 AD140150 AD140200	AD200050 AD200100 AD200150 AD200200
Linear Flow Rate (cm/h)	**Volumetric Flow Rate (ml/min)**			
25	16	33	64	131
50	32	65	128	262
75	48	98	192	393
100	64	131	257	524
120	77	157	308	628
150	96	196	385	785
200	128	262	513	1047
250	160	327	642	1309
300	193	393	770	1571

The flow rate table above provides a quick reference guide to determine the linear to volumetric flow rates of the respective Evolve® D column sizes. Please refer to the appropriate media user guide for the recommended flow rates.

Cleaning and Storage

Cleaning (column hardware)

The extensive solvent and chemical resistance of the column components allows the use of a wide range of cleaning and sanitisation solutions, including:

- Ethanol
- Peracetic acid (300 ppm)
- Iso-propyl alcohol (IPA)
- Hydrogen peroxide (HydroPure™)
- Sodium hydroxide (1.0 M)

Do not use abrasive cleaning materials or pads, as these may score the plastic components of the column.

Storage

For in-process storage, please consult the user guide of the resin packed within the column.

ORDER INFORMATION

Astrea Bioseparations can offer Evolve®D columns with 5, 10, 15 or 20 cm bed heights; for more information on this or any other supply related matters please do not hesitate to contact us on sales@astrea-bio.com

Appendix: Column Efficiency Test

1. Test the column at a flow rate of 100 cm/h. It is recommended to use a low to medium strength conductivity solution (e.g. 0.1 M NaCl) but will be dependent on the packed media.

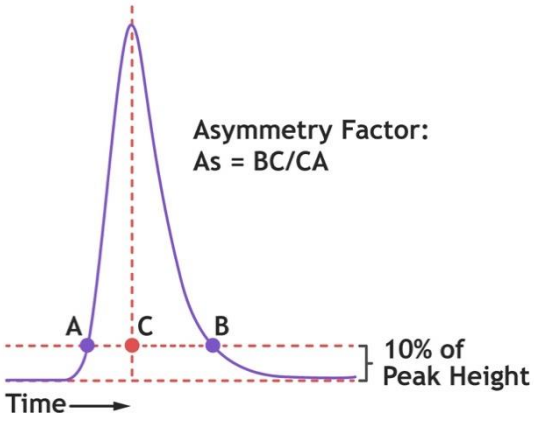
2. Attach the column to an equilibrated workstation.

3. Commence flow for 1 column volume (CV) to equilibrate and obtain a baseline.

4. Inject a 2% to 5% CV of a 1% to 2% acetone or 1 M to 2 M NaCl solution (dependant on packed media).

5. Continue the flow until a UV or conductivity peak (dependent upon tracer solution used) is observed and the trace has returned to baseline (~1.5 CV).

6. End run and determine the asymmetry factor:



7. To calculate the theoretical plate count (at half peak height), assuming a Gaussian peak:

   $N = 5.54 \times (Ve / W_{½})^2$

   Where:
   - N = number of theoretical plates
   - Ve = peak max (elution) volume (C) from tracer solution injection
   - $W_{½}$ = peak width at half height

8. Please refer to the associated documentation for the packed media with regards to the recommended specification for asymmetry and plate count/HETP.

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