High-throughput process development

PreDictor™ RoboColumn™ units

PreDictor RoboColumn units are miniaturized chromatographic columns prepacked with BioProcess™ chromatography resins from GE Healthcare Life Sciences (Fig 1). The columns are available for several chromatographic techniques such as ion exchange chromatography (IEX), affinity chromatography (AC), multimodal chromatography (MM), and hydrophobic interaction chromatography (HIC).

PreDictor RoboColumn units are part of the toolbox for high-throughput process development (HTPD). These miniaturized columns support HTPD by using a robotic liquid handling workstation, such as Freedom EVO™ from Tecan, for fully automated and parallel chromatographic separations. Perform HTPD work using PreDictor RoboColumn units alone or as a complement to PreDictor 96-well filter plates (Fig 2).
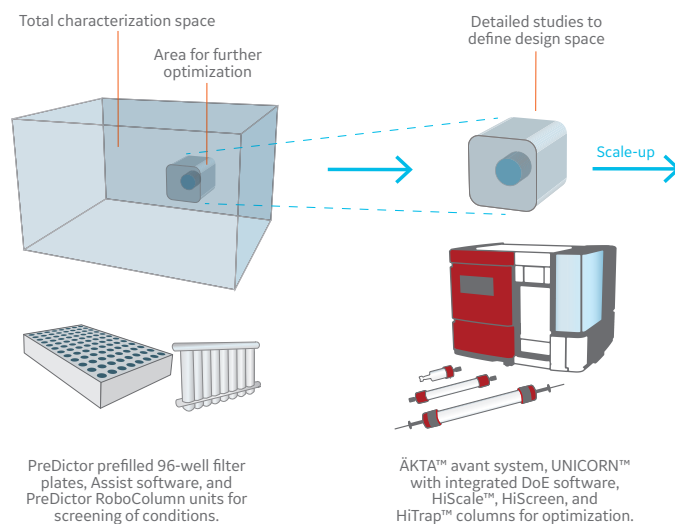
- Miniaturized column format: facilitates screening of a wide range of parameters with small sample consumption.
- Reduced experimental time: automation and parallel screening minimize manual labor and save time.
- Increased process understanding: high-throughput workflow allows investigation of enlarged experimental space for better process understanding.
- Support workflows using HTPD bioreactors with parallel purification for product quality comparisons.

Characteristics

PreDictor RoboColumn units are convenient screening tools, available in different volumes (50, 200, and 600 µL), and prepacked with a wide range of BioProcess chromatography resins. The columns are compatible with solutions commonly used in the purification of biopharmaceuticals. Cover seals at the top and bottom of each column prevent it from drying out. PreDictor RoboColumn units are supplied in rows of eight columns with the same chromatography resin, and can be individually and easily arranged on an array plate containing 96 positions. For PreDictor RoboColumn unit characteristics, see Table 1.

Fig 1. PreDictor RoboColumn units are prepacked miniaturized columns for fully automated chromatographic separation.

PreDictor prefilled 96-well filter plates, Assist software, and PreDictor RoboColumn units for screening of conditions.

ÄKTA™ avant system, UNICORN™ with integrated DoE software, HiScale™, HiScreen, and HiTrap™ columns for optimization.

Fig 2. Conceptual visualization of a workflow for process development. Parallel screening by using PreDictor plates or PreDictor RoboColumn units makes it possible to explore a large experimental space for optimal conditions. Once these have been identified, fine-tuning and verification are carried out on columns and ÄKTA systems. Finally, scale-up is done under conditions in which a robust production scale process can be operated.

Table 1. PreDictor RoboColumn* unit characteristics

Column

Column volume	50 µL	200 µL	600 µL
Bed height	2.5 mm	10.0 mm	30.0 mm
Column height	39 mm	39 mm	59 mm
Column i.d.	5 mm		
Column material	Polypropylene		
Filter material	Polypropylene		
Filter pore size	7 to 12 µm		
Chemical stability	Commonly used aqueous buffers, pH 1 to 14, organic solvents[†]		
Avoid	Halogenated organic solvents, hexane		
Storage solution	PreDictor RoboColumn Capto™ S ImpAct, Capto S, Capto SP ImpRes, Capto MMC ImpRes, SP Sepharose™ FF, SP Sepharose HP: 20% ethanol + 0.2 M sodium acetate All other PreDictor RoboColumn: 20% ethanol		
Recommended storage temperature	AIEX[‡], CIEX[‡], HIC, and MM: 4°C to 30°C AC: 2°C to 8°C		
Working temperature	4°C to 30°C		
Maximum working pressure[§]	Up to 8.0 bar		
Flow velocity	16 to 1000 cm/h		

Array plate

Plate size	128.3 × 14.0 × 86.0 mm (w × h × d)
Plate material	Polypropylene
Number of positions	96

* PreDictor RoboColumn units are packed by Atoll GmbH and are identical to Atoll GmbH RoboColumn units.

[†] Note: The chemical stability of the packed chromatography resins has also been taken into consideration. For more information, please refer to www.gelifesciences.com/bioprocess to find specifications for each chromatography resin.

[‡] AIEX = anion exchange, CIEX = cation exchange.

[§] Note: Please refer to the pressure limit for each chromatography resin.

HTPD workflow

PreDictor RoboColumn units can be used in a broad spectrum of applications, including parallel screening and optimization of chromatographic conditions as well as scale-down experiments. The columns are intended for automated workflows using a robotic liquid handling system such as Freedom EVO.

The HTPD workflow consists of the following steps:

1. Plan the experimental setup by using a systematic approach.
2. Perform the experiments, testing various experimental conditions in a parallel manner.
3. Analyze data using high-throughput analysis.
4. Evaluate data and analyze results.

Plan

The experimental setup should simultaneously test as many different conditions (factors) as possible. Preferentially, this is done using design of experiments (DoE), a statistical approach used to identify and define the factors having the greatest impact on the process or end product.

Using DoE, a maximum amount of information is obtained from a minimum number of experiments by varying the relevant factors simultaneously. The resulting DoE data (responses) are used to generate a statistical model that can predict the response to untested factor settings, which allows increased process understanding.

Before starting, the experimental objectives should be defined. Objectives may include:

Screening

- Which factors are most important (i.e., have a significant effect on the response)?
- What factor range should be explored?

Optimization

- What is the optimal combination of settings for the important factors?
- If there is more than one response, is it possible to find common factor settings that satisfy all responses, or is a compromise needed?

Robustness testing

- Is the process robust (i.e., unaffected typical process variations)?
- How much will the product characteristics vary due to process variations?

It is important to define the factors and responses that will be studied. The selection of analytical techniques for the responses will affect the amount of sample needed for each experiment. Running replicates is recommended for outlier analysis.

Perform

PreDictor RoboColumn units are designed for automated parallel experiments, minimizing manual work. The columns are supplied as a row of eight, although columns can easily be arranged individually. This modular design allows increased flexibility in designing and setting up experiments.

Analyze

HTPD experiments usually include a large number of samples, so high-throughput analytical methods are preferred if available. In some cases, simple absorbance measurements (e.g., at 280 nm) might be sufficient during initial screening. During the initial screening phase, analyses should be limited to the most critical responses. Based on the results, selected conditions can thereafter be chosen for responses that require more advanced analytical methods.

Evaluate

Data is evaluated, for example, by using traditional methods for determining resolution or breakthrough capacity. Based on the results, further investigation by using HTPD might be relevant before moving to larger columns for verification and fine-tuning.

Applications

Screening of chromatographic conditions

PreDictor RoboColumn units are miniaturized columns run in the same manner as conventional larger columns. These columns can be used to screen different parts of the chromatographic cycle such as binding studies (including dynamic binding capacities), elution studies, and wash studies. Figure 3 shows a screening experiment to determine which conditions give a high dynamic binding capacity (DBC). In Figure 4, binding capacity for conalbumin on Capto S resin is shown, using two different column formats (prepacked PreDictor RoboColumn units and user-packed Tricorn™ column) and a prepacked 96-well plate format (PreDictor plate).
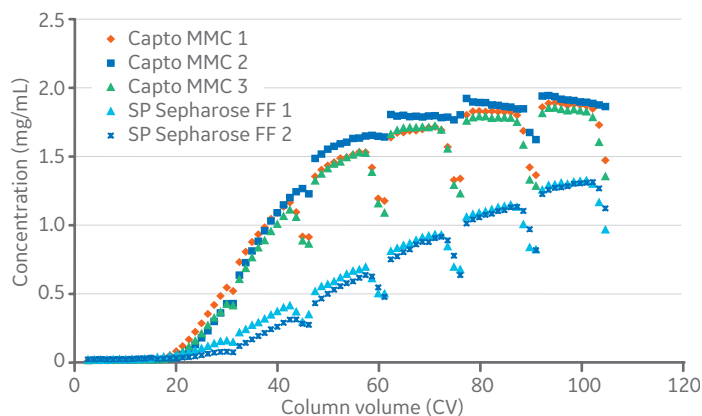
Figure 5 shows that PreDictor RoboColumn units give highly reproducible data, demonstrated in two different sets of DBC experiments.
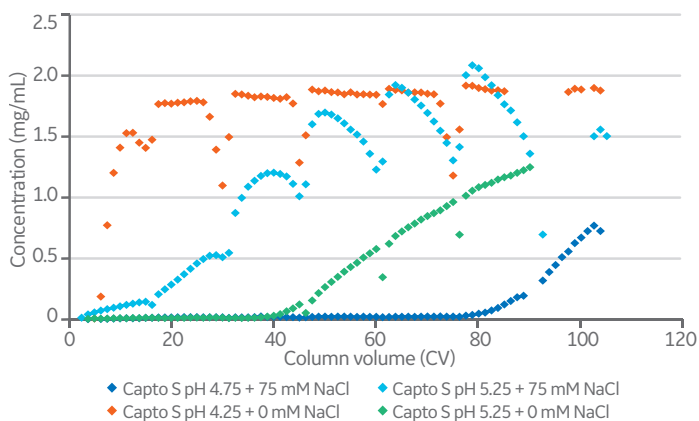
Fig 3. Breakthrough curves shown for conalbumin using PreDictor RoboColumn Capto S units, column volume 200 µL at different pH values and NaCl concentrations. Each data point represents a small fraction (droplet) from the column run.
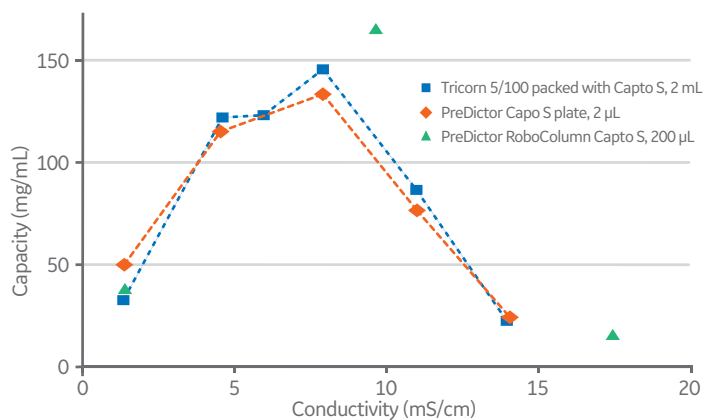
Fig 4. Binding capacity of Capto S resin for conalbumin at pH 4.75 shown for a 2 mL column (Tricorn 5/100), a 200 µL column (PreDictor RoboColumn), and 2 µL in batch mode (PreDictor plate). Capacities are shown as dynamic binding capacities (DBC) at 10% breakthrough for the two column formats and as static binding capacity (SBC) for the PreDictor Capto S, 2 µL plate. Fewer measurement points were measured for the PreDictor RoboColumn unit, however, the observed trend is similar.

Fig 5. High reproducibility shown for breakthrough curves from two different experiments using PreDictor RoboColumn units. One set of experiments is conalbumin on three different PreDictor RoboColumn Capto MMC units at pH 5.25 with 150 mM NaCl. The second set of experiments is conalbumin on two PreDictor RoboColumn SP Sepharose Fast Flow units, where breakthrough curves have been monitored at pH 4.75 with 75 mM NaCl.

Selectivity

In Figure 6, ribonuclease A, cytochrome c, and lysozyme were sequentially eluted using a step gradient consisting of NaCl at pH 7.2. A pseudochromatogram was plotted from the PreDictor RoboColumn data (Fig 6A). The data from using the miniaturized columns is similar to the results from the corresponding experiment using Tricorn 5/100 column (2 mL) shown in Figure 6B.
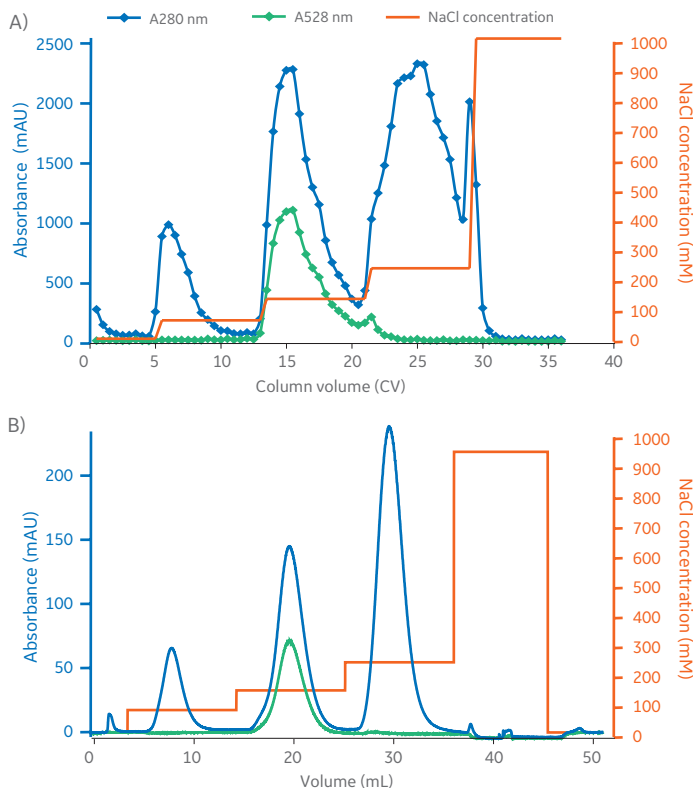
Fig 6. (A) Pseudochromatogram created by plotting absorbance and gradient versus column volume. Ribonuclease A, cytochrome c, and lysozyme were separated at pH 7.2 using PreDictor RoboColumn Capto S units, 200 µL. The elution of cytochrome c is easily detected at 528 nm as indicated by the orange curve. The extra peak observed at 30 CV is due to the short elution step. (B) Chromatogram showing the separation of ribonuclease A, cytochrome c, and lysozyme at pH 7.2 using a Tricorn 5/100 column packed with Capto S resin. The elution of cytochrome c is easily detected at 528 nm as indicated by the orange curve. The blue curve is the absorbance at 280 nm and the red curve is the NaCl gradient.

Ordering Information

List of selected PreDictor RoboColumn products. For additional requests please contact your local sales representative.

Note: pack size = one row of eight columns

Products		Product code
Ion exchange chromatography (IEX)		
PreDictor RoboColumn Capto S ImpAct	200 µL	17371771
	600 µL	17371772
PreDictor RoboColumn Capto Q	200 µL	28986072
	600 µL	28986175
PreDictor RoboColumn Capto S	200 µL	28986081
	600 µL	28986176
PreDictor RoboColumn Capto DEAE	200 µL	28986082
	600 µL	28986177
PreDictor RoboColumn Capto Q XP	200 µL	29275366
	600 µL	29275367
PreDictor RoboColumn Q Sepharose HP	200 µL	28986103
	600 µL	28986192
PreDictor RoboColumn SP Sepharose HP	200 µL	28986104
	600 µL	28986193
PreDictor RoboColumn Q Sepharose FF	200 µL	28986086
	600 µL	28986180
PreDictor RoboColumn SP Sepharose FF	200 µL	28986087
	600 µL	28986181
PreDictor RoboColumn CM Sepharose	200 µL	29275282
	600 µL	29275283
PreDictor RoboColumn Capto Q ImpRes	200 µL	28996918
	600 µL	28997391
PreDictor RoboColumn Capto SP ImpRes	200 µL	28997449
	600 µL	28997450
PreDictor RoboColumn Source 15 Q	200 µL	29275272
	600 µL	29275273
PreDictor RoboColumn Source 15 S	200 µL	29275274
	600 µL	29275275
PreDictor RoboColumn Source 30 Q	200 µL	29275276
	600 µL	29275277
Multimodal chromatography (MM)		
PreDictor RoboColum Capto MMC ImpRes	200 µL	17371640
	600 µL	17371641
PreDictor RoboColumn Capto adhere ImpRes	200 µL	17371540
	600 µL	17371541
PreDictor RoboColumn Capto MMC	200 µL	28986084
	600 µL	28986178
PreDictor RoboColumn Capto adhere	200 µL	28986085
	600 µL	28986179
PreDictor RoboColumn Capto Core 700	200 µL	29275362
	600 µL	29275363

Affinity chromatography (AC)		
PreDictor RoboColumn MabSelect™ PrismA	200 µL	17549833
	600 µL	17549834
PreDictor RoboColumn MabSelect SuRe™ pcc	200 µL	29275296
	600 µL	29275289
PreDictor RoboColumn MabSelect SuRe LX	50 µL	28986203
	200 µL	28986107
	600 µL	28997451
PreDictor RoboColumn MabSelect SuRe	200 µL	28997440
	600 µL	29093969
PreDictor RoboColumn MabSelect Xtra™	50 µL	28986202
	200 µL	28986106
PreDictor RoboColumn MabSelect	50 µL	28986204
	200 µL	29275290
	600 µL	29275291
PreDictor RoboColumn Capto L	200 µL	29003420
	600 µL	29003421
PreDictor RoboColumn Protein G Sepharose 4 FF	200 µL	29275288
	600 µL	29275289
PreDictor RoboColumn KappaSelect	200 µL	29275294
	600 µL	29275295
PreDictor RoboColumn MSS PCC	200 µL	29275296
	600 µL	29275297
PreDictor RoboColumn AVB Sepharose HP	200 µL	29275298
	600 µL	29275299
PreDictor RoboColumn LambdaFabSelet	200 µL	29275370
	600 µL	29275371
PreDictor RoboColumn VII Select	200 µL	29275278
	600 µL	29275279
PreDictor RoboColumn Capto Chelatin	200 µL	29275280
	600 µL	29275281
PreDictor RoboColumn Capto Blue	200 µL	29275364
	600 µL	29275365
PreDictor RoboColumn IMAC Sepharose 6FF	200 µL	29275368
	600 µL	29275369
PreDictor RoboColumn Capto DeVirS	200 µL	29275292
	600 µL	29275293

Hydrophobic interaction chromatography (HIC)

PreDictor RoboColumn Capto Phenyl (high sub)	200 µL	28986088
	600 µL	28986182
PreDictor RoboColumn Capto Butyl	200 µL	28986097
	600 µL	28986183
PreDictor RoboColumn Capto Octyl	200 µL	29275286
	600 µL	29275287
PreDictor RoboColumn Capto Butyl ImpRes	600 µL	17371941
PreDictor RoboColumn Capto Phenyl ImpRes	600 µL	17548441
PreDictor RoboColumn Phenyl Sepharose 6 FF(high sub)	200 µL	28986098
	600 µL	28986184
PreDictor RoboColumn Phenyl Sepharose 6 FF (low sub)	200 µL	28986099
	600 µL	28986188
PreDictor RoboColumn Butyl Sepharose 4 FF	200 µL	28986100
	600 µL	28986189
PreDictor RoboColumn Octyl Sepharose 4 FF	200 µL	29275284
	600 µL	29275285
PreDictor RoboColumn Butyl-S Sepharose 6 FF	200 µL	28986101
	600 µL	28986190
PreDictor RoboColumn Octyl Sepharose 4 FF	200 µL	28986102
	600 µL	28986191
PreDictor RoboColumn Butyl Sepharose HP	200 µL	28986173
	600 µL	28986195
PreDictor RoboColumn Phenyl Sepharose HP	200 µL	28986105
	600 µL	28986194

Accessories	Quantity	
96-well array plate for PreDictor RoboColumn units	1 plate	28986242
Microplate Foil, 96-well	100 × self-adhesive, transparent plastic foils	BR100578

Related Products	Quantity	
HiScreen Capto S ImpAct	1 × 4.7 mL	17371747
HiScreen Capto Q	1 × 4.7 mL	28926978
HiScreen Capto S	1 × 4.7 mL	28926979
HiScreen Capto DEAE	1 × 4.7 mL	28926982
HiScreen Capto Q ImpRes	1 × 4.7 mL	17547015
HiScreen Capto SP ImpRes	1 × 4.7 mL	17546815
HiScreen Q HP	1 × 4.7 mL	28950511
HiScreen SP HP	1 × 4.7 mL	28950515
HiScreen Q FF	1 × 4.7 mL	28950510
HiScreen DEAE FF	1 × 4.7 mL	28978245
HiScreen SP FF	1 × 4.7 mL	28950513
HiScreen Capto MMC ImpRes	1 × 4.7 mL	17371620
HiScreen Capto adhere ImpRes	1 × 4.7 mL	17371520
HiScreen Capto MMC	1 × 4.7 mL	28926980
HiScreen Capto adhere	1 × 4.7 mL	28926981
HiScreen MabSelect PrismA	1 × 4.7 mL	28926973
HiScreen MabSelect SuRe LX	1 × 4.7 mL	17549815
HiScreen MabSelect SuRe	1 × 4.7 mL	28926977
HiScreen MabSelect Xtra	1 × 4.7 mL	28926976
HiScreen MabSelect	1 × 4.7 mL	17547415
HiScreen Capto L	1 × 4.7 mL	17547814
HiScreen Capto Phenyl (high sub)	1 × 4.7 mL	28992472
HiScreen Phenyl HP	1 × 4.7 mL	28950516
HiScreen Phenyl FF (high sub)	1 × 4.7 mL	28926988
HiScreen Phenyl FF (low sub)	1 × 4.7 mL	28926989
HiScreen Capto Butyl	1 × 4.7 mL	28992473
HiScreen Butyl HP	1 × 4.7 mL	28978242
HiScreen Butyl FF	1 × 4.7 mL	28926984
HiScreen Butyl-S FF	1 × 4.7 mL	28926985
HiScreen Octyl FF	1 × 4.7 mL	28926986

Related literature

Title	Product code
Instructions	
PreDictor RoboColumn	28987015
Data files	
PreDictor 96-well filter plates and Assist software	28925839
HiScreen prepacked columns	28930581
Capto S ImpAct	29067018
Capto S, Capto Q and Capto DEAE	11002576
Capto SP ImpRes and Capto Q ImpRes	28983763
Capto MMC ImpRes	29035674
Capto MMC	11003545
Capto adhere ImpRes	29034497
Capto adhere	28907888
MabSelect PrismA	18114994
MabSelect SuRe LX	KA553200917DF
MabSelect SuRe	11001165
MabSelect Xtra	11001157
MabSelect	28987062
Capto L	29010008
Capto Butyl, Capto Phenyl (high sub)	28955857
Butyl Sepharose 4 FF	18102070
Phenyl Sepharose 6 FF (low sub) and Phenyl Sepharose 6 FF (high sub)	18102053
Sepharose Fast Flow IEX media and prepacked formats	18102066
Butyl-S Sepharose 6 FF	11002634
Butyl Sepharose HP and Phenyl Sepharose HP	18117287

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