



# Capto S ImpAct

## Ion Exchange Media

### Instructions for Use

Capto™ S ImpAct is a strong cation exchange BioProcess™ chromatography medium (resin) for intermediate purification and polishing of a wide range of biomolecules, especially monoclonal antibodies. The high binding capacity and the high flow base matrix in combination with the small particle size of Capto S ImpAct results in excellent pressure-flow properties as well as impressive resolution.

Capto S ImpAct provides:

- Efficient aggregate removal at high load of monoclonal antibodies
- High-resolution intermediate purification and polishing based on the well-established Capto platform with novel polymeric ligand
- Flexibility of design – large operational window of flow rates and bed heights
- High-throughput purifications easy to optimize and scale up
- Higher manufacturing productivity enables improved process economy
- Security of supply and comprehensive regulatory support

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Read these instructions carefully before using the products.

## **Safety**

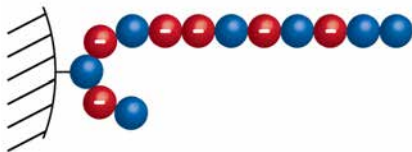
For use and handling of the products in a safe way, refer to the Safety Data Sheets.

# 1 BioProcess resins

BioProcess media are developed and supported for production scale chromatography. All BioProcess media are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

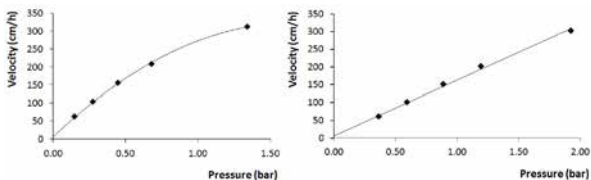
## 2 Properties of Capto S ImpAct

The structure of the well established S (sulfonate group) ligand used for Capto S ImpAct is shown in Figure below.



**Fig 1.** Sketch showing the strong ion exchange group of Capto S ImpAct

The medium is designed for intermediate purification or polishing. It is based on a high flow agarose base matrix, which gives good pressure-flow properties (following Figure).



**Fig 2.** Example of pressure flow curves for Capto S ImpAct in open bed (left) and packed bed (right), respectively. Running conditions: AxiChrom™ 300 (30 cm i.d.), 20 cm bed height with Packing factor 1.20 in 0.15 M NaCl at 20°C. Pressure contribution from system, tubings and column is excluded.

The small bead size of Capto S ImpAct allows for high resolution purifications. The combination of the well established S ligand with a small high flow agarose bead makes the medium ideal for intermediate purification and polishing. Further characteristics of the medium are found in the following Table.

**Table 1.** Characteristics of Capto S ImpAct

<b>Matrix</b>	High flow agarose
<b>Functional group</b>	SO <sub>3</sub> <sup>-</sup>
<b>Total ionic capacity</b>	37 to 63 µmol (H <sup>+</sup> ) /mL medium
<b>Average particle size (d<sub>50v</sub>)<sup>1</sup></b>	50 µm
<b>Maximum operational flow velocity<sup>2</sup></b>	At least 220 cm/h in a 1 m diameter column with bed height 20 cm at 20°C; measured using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
<b>Binding capacity<sup>3</sup> (mg/mL medium)</b>	> 90 mg lysozyme > 85 mg BSA > 100 mg IgG
<b>pH stability<sup>4</sup></b>	
Working	4 to 12
Cleaning-in-place	3 to 14
<b>Working temperature</b>	4°C to 30°C
<b>Chemical stability</b>	All commonly used aqueous buffers, 1 M sodium hydroxide <sup>5</sup> , 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol
<b>Avoid</b>	Oxidizing agents, cationic detergents
<b>Storage</b>	0.2 M sodium acetate in 20% ethanol

<sup>1</sup> d<sub>50v</sub> is the median particle size of the cumulative volume distribution.

<sup>2</sup> Flow velocity stated in the table is dependent on the column used.

<sup>3</sup> Dynamic binding capacity at 10% breakthrough measured at a residence time of 5.4 minutes in a Tricorn™ 5/50 column with 5 cm bed height (corresponding to 220 cm/h in a 20 cm bed height column).

25 mM sodium phosphate, pH 7.2 (lysozyme), 50 mM sodium acetate, pH 5.0 (BSA) and 50 mM sodium acetate, pH 5.5 (IgG)

<sup>4</sup> Working range: pH interval where the medium can be operated without significant change in function.

Cleaning-in-place: pH stability where the medium can be subjected to cleaning-in-place without significant change in function.

<sup>5</sup> No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

### 3 Method optimization

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. To save sample and time it is recommended to optimize the design of the method in laboratory scale.

Elution of protein can either be done by use of salt, pH or a combination of both. For optimization of the elution, sample load, flow velocity and gradient volume should be considered. The three factors are interrelated and best results will be obtained using:

- Maximized sample load with respect to dynamic binding capacity.
- Maximized flow velocity with respect to system constraints and media rigidity.
- The gradient volume that provides the best resolution with maximized sample load and maximized flow velocity.

The use of PreDicator™ plates is preferentially included in the method development. The PreDicator plates are 96-well filter plates pre-filled with chromatography media, which can be used for rapid screening of chromatographic conditions in small scale. The suggested workflow with PreDicator plates is shown in the following Figure, where a large design space can be explored prior to further experiments in packed column formats, such as prepacked HiScreen™ columns.



**Fig 3.** The recommended workflow is described in the figure. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.

**Table 2.** The experimental conditions to consider when designing and optimizing the process.

Phases	Activity	Conditions to consider
1. Equilibration of column and sample preparation	Equilibration of column and adjustment of sample	<ul style="list-style-type: none"> <li>• pH</li> <li>• Conductivity</li> <li>• Column volume</li> <li>• Column bed height</li> <li>• Particle content</li> <li>• Temperature</li> </ul>
2. Sample application	Manual or automatic application onto the column	<ul style="list-style-type: none"> <li>• Flow rate</li> <li>• Sample pH</li> <li>• Sample conductivity</li> <li>• Upward/downward flow</li> </ul>
3. Wash	Wash out unbound material with clean binding buffer	<ul style="list-style-type: none"> <li>• Flow rate</li> <li>• Upward/downward flow</li> <li>• Buffer choice (normally same as column equilibration buffer)</li> </ul>

Phases	Activity	Conditions to consider
4. Elution	Elute the material from the column either with salt or by change in pH	<ul style="list-style-type: none"> <li>• Sample load</li> <li>• pH</li> <li>• Conductivity</li> <li>• Flowrate</li> <li>• Upward/downward flow</li> </ul>

For more information about method development and optimization, consult *Handbook 11000421* and *Handbook 28940358*.



## 4 Scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the linear flow velocity (cm/h) applied during sample loading.

### Procedure

Step	Action
------	--------

- |   |  |
|---|--|
| 1 | Select bed volume according to required binding capacity. Keep sample concentration and gradient slope constant. |
| 2 | Select column diameter to obtain the bed height (10 to 40 cm) from method optimization.                          |

**Note:**

*The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.*

Step	Action
------	--------

- |   |   |
|---|---|
| 3 | The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems. Check also the compatibility of the hardware and chromatography medium pressure limits with expected pressure during packing and operation. |
|---|---|

## 5 Column packing

### Packing Tricorn columns

The following instructions are for packing Tricorn 5/100 and Tricorn 10/100 columns with a 10 cm bed height.

For more details about packing Tricorn columns, see *Instructions 28409488*.

### Packing preparations

#### Materials

- Capto S ImpAct
- Plastic spoon or spatula
- Glass filter G4
- Vacuum suction equipment
- Filter flask
- Measuring cylinder

- Thin capillary or glass rod
- 0.4 M NaCl
- Tricorn 5/100 column, Tricorn Glass Tube 5/100 (to be used as packing tube), and Tricorn Packing Connector 5-5, or
- Tricorn 10/100 column, Tricorn Packing Equipment 10/100, which includes the 10-mm packing connector, 100-mm glass tube (to be used as packing tube).
- Bottom unit with filter holder, filter, cap, and stop plug.

## Equipment

Chromatography system, such as ÄKTA™ system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

## Washing the chromatography medium

Mount the glass filter funnel onto the filtering flask. Suspend the medium by shaking and pour into the funnel and wash according to the following instructions:

Step	Action
1	Wash 5 times with 5-10 mL 0.4 M NaCl/mL chromatography medium.
2	Gently stir with a spatula between additions.
3	Move the washed medium from the funnel into a beaker and add 0.4 M NaCl to obtain a 50% slurry concentration.

## Preparing the packing slurry

Check the slurry concentration after settling overnight in a measuring cylinder or use the method for slurry concentration measurement described in *Application note 28925932*.

Tricorn columns can be packed with an excess of chromatography medium to be removed after packing.

## Packing procedure

### Main features

**Table 3.** Main features of the packing method.

	Tricorn 5/100	Tricorn 10/100
Slurry/packing solution	0.4 M NaCl	0.4 M NaCl
Slurry concentration (%)	50	50
<b>Phase 1</b>		
Packing velocity (cm/h)	1070	1070
Packing flow (mL/min)	3.5	14.0
Packing time (min)	10	10
<b>Phase 2</b>		
Packing velocity (cm/h)	1070	1070
Packing flow (mL/min)	3.5	14.0
Packing time (min)	2	2

## Procedure

### Preparing packing

Step	Action
------	--------

- |   |   |
|---|---|
| 1 | Assemble the column according to <i>Instructions 28409488</i> . For additional information, please visit Technical support at <a href="https://www.cytiva.com/tricorn">cytiva.com/tricorn</a> . |
|---|---|

Step	Action
------	--------

- |   |  |
|---|--|
| 2 | Put a stop plug in the bottom of the column tube and pour the suspended medium slurry (50%) into the top of the packing tube, filling both column tube and packing tube. Avoid formation of air bubbles in the medium by pouring it along a thin capillary or glass rod. |
| 3 | Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column tube and connect a pump to the top of the packing unit. Remove the stop plug from the bottom of the column tube.  |

## Phase 1

Step	Action
------	--------

- |   |   |
|---|---|
| 1 | Pack the medium at 3.5 mL/min (Tricorn 5/100) or 14.0 mL/min (Tricorn 10/100) for 10 minutes.   |
| 2 | When the medium is packed, switch off the pump, attach the stop plug into bottom of the column tube, disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess medium by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula. |
| 3 | Fill up the column with the same solution that was used for packing the column.   |

Step	Action
------	--------

- |   |  |
|---|--|
| 4 | Place a pre-wetted filter on top of the solution in the column and gently push it into the column tube with the filter tool. |
|---|--|

**Note:**

*Coarse filter should not be used with Capto S ImpAct.*

- |   |   |
|---|---|
| 5 | Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then turn it back 1.5 turns.   |
| 6 | Wet the O-ring on the adapter unit by dipping it into water or packing solution.  |
| 7 | Screw the adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Ensure that there are no trapped air bubbles. |
| 8 | Screw the adapter down to approximately 1 mm above the surface of the bed.  |
| 9 | Connect the pump to the adapter unit. Remove the stop plug in the bottom of the column.   |

## Phase 2

Step	Action
------	--------

- |   |  |
|---|--|
| 1 | Pack the medium at 3.5 mL/min (Tricorn 5/100) or 14.0 mL/min (Tricorn 10/100) for 2 minutes. |
| 2 | Mark the bed height on the column tube with a pen.   |

Step	Action
3	Switch off the pump, attach the stop plug into bottom of the column tube and disconnect the pump slowly.
4	Turn the adapter down to the mark.
5	Press the adapter lock down into the locked position.
6	Screw a stop plug into the adapter unit. The column is now ready to be used.

### Testing the packed column

See section *Evaluation of column packing*.

## Packing HiScale columns

### Introduction

The following instructions are for packing HiScale™ 16, 26 and 50 with 10 to 35 cm bed height. For more details about packing HiScale columns, see *Instructions 28967470*.

### Materials needed

- Capto S ImpAct
- HiScale column
- HiScale packing tube (depending on bed height)
- Measuring cylinder
- 20% ethanol with 0.2 M sodium acetate

## Equipment

- Chromatography system, such as ÄKTA system, or a standalone pump such as Pump P-900, depending on the flow rate required, can be used for packing.
- Pressure monitor

Equilibrate all materials to room temperature.

## Preparation of the slurry

To measure the slurry concentration, let the medium settle in 20% ethanol with 0.2 M sodium acetate at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in *Application note 28925932*.



## Packing parameters

**Table 4.** HiScale 16

Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.2 M sodium acetate		
Slurry concentration (%)	45 - 55		
Step 1			
Consolidation velocity (cm/h)	300	220	100
Consolidation flow (mL/min)	10.1	7.4	3.3
Consolidation time (min)	20	20	30
Step 2			
Packing velocity (cm/h)	750	500	250
Packing flow (mL/min)	25.1	16.7	8.4
Packing time (min)	20	20	20

**Table 5.** HiScale 26

Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.2 M sodium acetate		
Slurry concentration (%)	45 - 55		
Step 1			
Consolidation velocity (cm/h)	300	220	100
Consolidation flow (mL/min)	27	19	9
Consolidation time (min)	20	20	30
Step 2			
Packing velocity (cm/h)	600	400	250
Packing flow (mL/min)	53	35	22
Packing time (min)	20	20	20

**Table 6.** HiScale 50

Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.2 M sodium acetate		
Slurry concentration (%)	45 - 55		
Step 1			

Bed height (cm)	10	20	35
Consolidation velocity (cm/h)	300	220	100
Consolidation flow (mL/min)	98	72	33
Consolidation time (min)	20	20	30
<b>Step 2</b>			
Packing velocity (cm/h)	600	300	200
Packing flow (mL/min)	196	98	65
Packing time (min)	20	20	20

## Packing the column

### Step Action

- 1 Mount the packing tube at the top of the column.
- 2 Wet the bottom filter by injecting 20% ethanol with 0.2 M sodium acetate through the effluent tubing and mount filter and bottom piece on the column.
- 3 Mount the column and packing tube vertically on a laboratory stand.
- 4 Apply 20% ethanol with 0.2 M sodium acetate 2 cm over the column bottom adapter and put a stop plug on the outlet.
- 5 Pour the medium slurry into the column and packing tube and if necessary top up carefully with 20% ethanol with 0.2 M sodium acetate.
- 6 Connect the top adapter to the pump and prime the top adapter with packing solution.

Step	Action
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7	Mount the top adapter in the packing tube, sliding it down to the surface of the slurry and displacing the air under the adapter.
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8	Pack the column with 20% ethanol with 0.2 M sodium acetate at a constant flow (see Tables in Section <a href="#">Packing parameters, on page 17</a> , Step 1) and run for 20-30 min or until the medium bed is stable.
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9	Increase the flow (Tables in Section <a href="#">Packing parameters, on page 17</a> , Step 2) and run for 20 minutes.
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**Note:**

*If too high back pressure is obtained in the second packing step, try re-packing at a lower flow.*

10	Register the bed height on the column scale.
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11	Stop the pump, close the column outlet and dismount the packing tube (if used).
----	---

12	Mount the adapter in the column tube and adjusted it down to approximately 2 cm above the bed surface with the o-ring untightened.
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13	Tighten the o-ring and adjust the adapter down to the bed height noted in Step 10 with the inlet on top of the column open.
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If too high back pressure is obtained in the second packing step, try re-packing at a lower flow.

## Testing the packed column

See Section *Evaluation of column packing*.

# 6 Evaluation of column packing

## Intervals

Test the column efficiency to check the quality of packing. Testing should be done after packing and at regular intervals during the working life of the column and also when separation performance is seen to deteriorate.

## Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor ( $A_s$ ). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult *Application note 28937207*.

**Note:** *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

## Sample volume and flow velocity

For optimal results, the sample volume should be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

## Method for measuring HETP and $A_S$

Calculate HETP and  $A_S$  from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

$L$  = bed height (cm)

$N$  = number of theoretical plates

$V_R$  = volume eluted from the start of sample application to the peak maximum.

$$N = 5.54 \times \left( \frac{V_R}{W_h} \right)^2$$

$W_h$  = peak width measured as the width of the recorded peak at half of the peak height.

$V_R$  and  $W_h$  are in the same units.

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height,  $h$ , is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

$d_{50v}$  = mean diameter of the beads (cm)

As a guideline, a value of  $< 3$  is very good.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (typical acceptable range could be  $0.8 < A_S < 1.8$ ).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

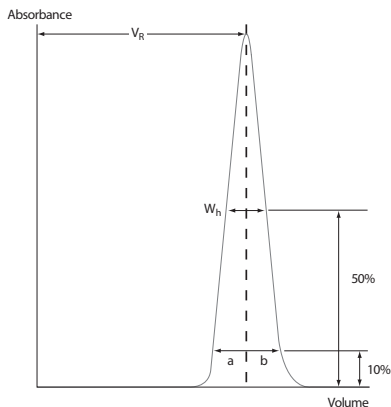
Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

The Figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and  $A_s$  values are calculated.



**Fig 4.** A typical test chromatogram showing the parameters used for HETP and  $A_s$  calculations.

## 7 Maintenance

For best performance from Capto S ImpAct and to maximize the life time of the medium, follow the procedures described below.

### Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with five bed volumes or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with start buffer until the conductivity and pH values are stable.

### Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1 to 2 M NaCl in elution buffer). Regenerate the medium by washing with five bed volumes of start buffer or until the column effluent shows stable conductivity and pH values.

### Cleaning-in-place (CIP)

Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain capacity, flow properties and general performance.

A specific CIP protocol should be designed for each process according to the type of contaminants present.

Precipitated, hydrophobically bound proteins or lipoproteins

Wash with 1 M NaOH solution with reversed flow direction. Contact time 15 to 30 min (sample dependent). Use 1 M NaOH with 1 M NaCl if the results are not satisfactory with only NaOH.

Ionically bound proteins	Wash with 0.5 to 2 column volumes of 2 M NaCl with reversed flow direction. Contact time 10 to 15 minutes.
Lipids and very hydrophobic proteins	Wash with 2 to 4 column volumes of up to 70% ethanol <sup>1</sup> or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h. Alternatively, wash with 2 to 4 column volumes of 0.1 to 0.5% non-ionic detergent with reversed flow direction. Contact time 1 to 2 h.

<sup>1</sup> Specific regulations may apply when using 70% ethanol since the use of explosion-proof areas and equipment may be required.

## Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 h is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins sanitizes the medium effectively.

## Storage

Capto S ImpAct: 0.2 M sodium acetate in 20% ethanol.  
Storage temperature: 4°C to 30°C.

Store unused media in the container at a temperature of 4°C to 30°C. After storage, equilibrate with at least five column volumes of start buffer.

# 8 Ordering information

Product	Quantity	Code No
CaptoS ImpAct	25 mL	17371701
	100 mL	17371702



Product	Quantity	Code No
	1 L	17371703
	5 L	17371704
	10 L	17371705
	60 L	17371760

Capto S ImpAct is supplied as a suspension in 20% ethanol containing 0.2 M sodium acetate. For additional information, please contact your local Cytiva representative.

## Related products

Product	Quantity	Code No
HiTrap™ Capto S ImpAct	5 x 1 mL	17371751
HiTrap Capto S ImpAct	5 x 5 mL	17371755
HiScreen Capto S ImpAct	1 x 4.7 mL	17371747
PreDictor Capto S ImpAct, 2 µL	4 × 96-well filter plates	17371716
PreDictor Capto S ImpAct, 20 µL	4 × 96-well filter plates	17371717
PreDictor RoboColumn™ Capto S ImpAct, 200 µL	8 columns in row	17371771
PreDictor RoboColumn Capto S ImpAct, 600 µL	8 columns in row	17371772
PreDictor Capto CIEX screening, 2 µL/6 µL	4 × 96-well filter plates	29095568
PreDictor Capto CIEX screening, 20 µL	4 × 96-well filter plates	29095567
<b>Empty columns</b>		
Tricorn 5/100	1	28406410
Tricorn 10/100	1	28406415
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513

Product	Quantity	Code No
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

## Accessories

Product	Quantity	Code No
Tricorn Glass Tube 5/100	1	18115306
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
Packing tube 20 (HiScale 16)	1	28986816
Packing tube 40 (HiScale 16)	1	28986815
Packing tube 20 (HiScale 26)	1	28980383
Packing tube 40 (HiScale 26)	1	28964505
Packing tube 20 (HiScale 50)	1	28980251
Packing tube 40 (HiScale 50)	1	28964506

## Literature

Product	Code No
Data File: Capto S ImpAct	29067018
Application Note: Methods for packing Capto S, Capto Q and Capto DEAE in production scale columns	28925932
Application Note: Column efficiency testing	28937207
Guide: Comparison guide for process development tools	28995164
Handbook: Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11000421
Handbook: High throughput process development with PreDicator plates	28940358
Instructions: Tricorn Empty High Performance Columns	28409488
Instructions: HiScale columns (16, 26, 50) and accessories	28967470

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