User Guide

# OPUS® 1.2 – 2.5 cm Diameter Columns Pre-packed with CaptivA® Resin


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INSPIRING ADVANCES IN BIOPROCESSING

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## Contents



NOTE: This User Guide is specific for OPUS® 1.2 – 2.5 cm diameter columns packed with CaptivA® resin. OPUS® columns packed with other chromatography medias will vary in performance characteristics, method screening, processing, maintenance and storage.

## Safety Notices:

- Please review the Material Safety Data Sheet for this product prior to using it
- This product is shipped in 18.5% ± 1% ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use.
- Follow all local regulations for safe disposal
- For laboratory and manufacturing production use only
- Not for administration to humans

## Introduction

OPUS® columns pre-packed with CaptivA® resin are designed for the purification of biopharmaceuticals. OPUS® columns are equally suited for process development as well as clinical and smaller scale commercial manufacturing. The columns are manufactured with materials that are well characterized, including Nylon 6-6, polypropylene (PP), and polyethylene (PE) and are available in a range of diameters and bed heights. These columns are compatible with any HPLC, FPLC™ or AKTA™ system.

CaptivA® resin is the first Protein A affinity chromatography media designed to significantly reduce cost of investment by offering excellent performance, high capacity, high flow rate, and low leaching for a fraction of the cost of traditional resins. CaptivA® resin combines an industry standard cross-linked agarose bead with recombinant native Staphylococcal Protein A (rSPA), manufactured by Repligen, the world' s leading manufacturer of recombinant Protein A for over 20 years.

## CaptivA® Resin Characteristics

| Property | Value |
|---|---|
| Matrix Composition | 4% Highly cross linked Agarose |
| Ligand | Recombinant Protein A (rSPA) Animal Free |
| Particle Size | 45 – 165 µm |
| Coupling Chemistry | Multi-Point Attachment via Reductive Amination |
| Recommended Working Velocity | 30 – 300 cm/hr |
| Temperature Stability | 2 - 40°C<br>Long Term Storage: Store at 2-8°C |
| Delivery Conditions | Shipped RT, 52±1% Slurry Containing 18.5 ± 1% Ethanol |
| Recommended pH:<br>Working<br>Clean in Place | <br>3 – 10<br>2 – 11 |
| Storage Conditions | 2-8°C in the presence of a bacteriostatic agent<br>(e.g. 18.5 - 20% Ethanol)<br>**Protect from freezing** |
| Binding Capacity | Static: >40 mg human IgG/ml resin<br>Dynamic: Binding of antibody to CAPTIVA® resin may be end user specific thus determination must be made on a process/product specific basis |
| Leachable Protein A | ≤ 5 ng rSPA per milliliter resin |
| Recommended pH working range | 3 – 10 |
| Regeneration | After each separation cycle, regenerate the CaptivA® resin bed by washing with 3CVs of 0.1 M Acetic Acid or 0.1 M Phosphoric Acid |

## Column Characteristics

| Size | Components | Connections | Max. Back Pressure |
|---|---|---|---|
| 1.2 cm x 10 cm 11.3 mL | Column Body Polypropylene Unitary Ends –Nylon 6-6; 10 µm Titanium Frit | ¼-28 Threaded Flat Bottom Ports ¼-28 Flangeless Ferrule and Nut for connection to 1/16" OD tubing included | 3-5 Bar |
| 1.2 cm x 20 cm 23 mL | | | |
| 2.5 cm x 10 cm 49 mL | Column Body — Polyethylene (MOPE) Unitary Ends — Nylon 6-6 with 5 µm Nylon mesh | ¼ -28 Threaded Flat Bottom Ports ¼ -28 Flangeless Ferrule and Nut for connection to 1/16" OD tubing included | 3-5 Bar |
| 2.5 cm x 20 cm 98 mL | | | |

- Working Temperature range is 4 – 30°C
- Storage Solution 2/3 PBS, 18.5% EtOH
- Chemical compatibility with most typical chromatographic buffers & solvents including:
  - Aqueous buffers and salt solutions

8M Urea
6M Guanidine
25% Ethanol
20% Isopropanol

Conversion from linear to volumetric flow rates:

| Column Diameter | Flow at 100 cm/hr | Flow at 200 cm/hr | Flow at 300 cm/hr |
|---|---|---|---|
| 1.2 cm | 1.9 mL/min | 3.8 mL/min | 5.7 mL/min |
| 2.5 cm | 8.2 mL/min | 16.4 mL/min | 24.6 mL/min |

# Operation

## Chromatography System Set Up

### System Pressure

*Note: It is recommended to set the system pressure alarm at 5 bar (75 psi, 0.5 MPa) to provide an appropriate safety factor. Failure to set a pressure alarm could result in pressures that could compromise the media and/or column structure.*

Typical operating pressures for a column packed with CaptivA® resin are usually < 3 bar. The systems should be used with a back-pressure regulator to prevent degassing in the detector flow cell following the system manufacturer's instructions.

All buffers or feedstock solutions being applied to the column should be 0.22 µm filtered. Include a 2 µm in-line filter (supplied with AKTA™ system) in the chromatography system upstream of the column.

### Column System Connection

OPUS® 1.2 cm & 2.5 cm diameter columns both have identical, ¼-28 threaded flat bottom inlet and outlet ports.

For 1/4-28 Threaded Connections:

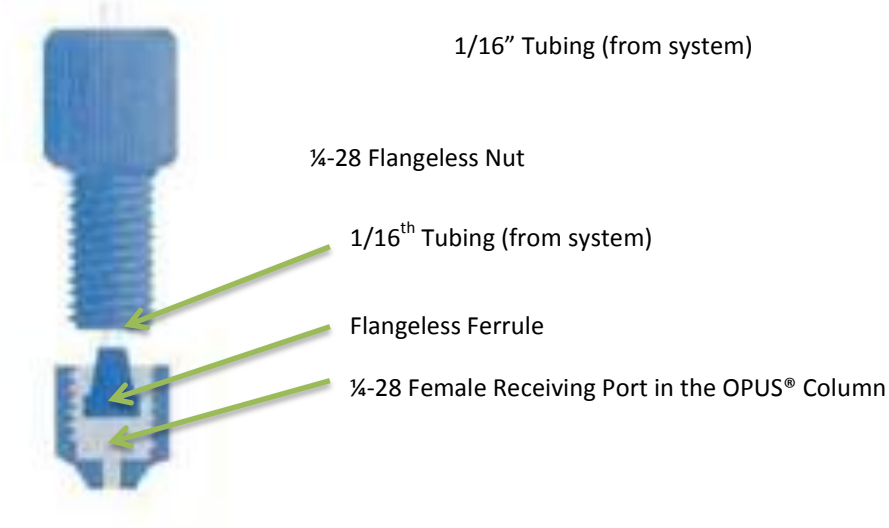
1. Remove the upper stopper from the column and attach a suitable /-4-28 threaded fitting to the OPUS® column. The connection should be made "drop-to-drop" to avoid introduction of air into the system. Finally turn the /-4-28 threaded fitting connector nut clockwise until it is finger tight. Do not to over tighten the fitting.
2. Remove the bottom stopper from the OPUS® column and repeat the connection process described above, connecting the -28 threaded outlet to the OPUS® column to the chromatography system.
3. CaptivA® resin is preserved in an 18.5 ± 1% ethanol solution; before startup the column should be flushed with at least 5 column volumes of R0/01 water or mild buffer (e.g. PBS) at 100 em/hr. Finally equilibrate the column by following the initial column flush with a second flush of 2-5 cv's of the desired equilibration buffer. Other resins should be rinsed and equilibrated according to the manufacturer's recommendations.

For 1/16" Tubing Connection:

*WARNING: When used directly with a 1/16" tubing connection (e.g. an AKTA System) the 1/4-28 OPUS® column threads may leak.*

To avoid leaks when using a 1/16" tubing connection each 1.2 cm and 2.5 cm diameter OPUS® column is supplied with a pair of –28 flangeless ferrule fittings manufactured by Upchurch (Fig 1.) Scientific® for connecting to 1/16" OD tubing (Catalog numbers P-200x and P-210N)

Fig 1. Upchurch Scientific® 1/16" OD Tubing Connector




## Instructions for Installing Upchurch Scientific® 1/16" OD Tubing Connectors

To minimize the risk of leaks resulting from improper connections, please follow these directions when connecting the 1/16th system tubing into the threaded flat-bottom receiving port of the column.[1]

1. For polymer tubing, cut the end of the tubing, leaving a square-cut face. If other tubing materials are being used (e.g. steel or other metallic tubing) ensure the end of the tubing is flat and burr-free.
2. Slide the Flangeless nut over the tubing, with the nut threads facing the tubing end being connected.
3. Slip the Flangless ferrule over the tubing, with the tapered portion of the ferrule facing towards the nut. Align the end of the tubing such that it just projects through the ferrule, slide the tubing and ferrule back so it touches the bottom of the flangeless nut.
4. Insert the flangeless nut, tubing, ferrule assembly in place into the ¼ -28 female receiving port of the OPUS® column, and while holding the tubing down firmly into the port, tighten the nut finger tight. The action of tightening the flangeless nut seals the ferrule to both the tubing and the nut.

NOTE: If you experience difficulty connecting the column to your system, please contact Technical Service at 800-622-2259 for further assistance.

Once installation of the Upchurch Scientific© connectors are complete continue and follow the set up instruction outlined in 2 & 3 for the -28 threaded connectors above.

[1] Instructions For Use, Flangeless Fittings, IDEX Health & Sciences LLC – Home of Upchurch Scientific Products, P01 11/09

## Method Screening

CaptivA® resin affinity varies for antibodies of different species, classes and subclasses, initial screening should be conducted under a broader range of conditions that will bind the largest diversity of antibodies and highlight potential interference between the target antibody and possible contaminating antibodies.

A good general approach to evaluating primary mAb binding to CaptivA® resin is to start with a moderately alkaline pH and high salt conditions, then elute them in a reducing linear saiUpH gradient. It is important to make certain that the antibody is stable under the elution conditions in order to avoid loss of biological activity or column precipitation which may also lead to column fouling.

General Screening Recommendations
Example of suitable buffers:

- Buffer A: 0.05 M Tris buffer, 1.0 to 2.0 M NaCl, pH 8.5
  OR PBS pH 7.4
- Buffer B: 0.05 M sodium citrate, 0.3 M NaCl, pH 3.0
  OR 0.1M Glycine/HCL, pH 3.0-3.5

*Note: The use of high concentrations of salt* has *been known to increase IgG binding capacity for IgG from species with lower Protein A affinity.*

Experimental conditions:

- Equilibrate the column with 10 column volumes of buffer A
- Apply a small sample of antibody
- Wash the column with 5 column volumes of buffer A
- Elute the column with a linear gradient of 10 column volumes to 100% buffer B
- Collect fractions into titrating diluent (e.g. 1.0 M Tris-Base, pH 8.0 so that the diluent volume equals 5% of the programmed fraction volume)
- Regenerate the column with 5–10 column volumes of 100% buffer B
- Re-equilibrate the column with buffer A

Conditions can be subsequently modified to provide the best purification performance.

## Processing and Maintenance

### Cleaning-in-place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the resin and hardware. The accumulation of these contaminants may affect subsequent performance of the purification system or allow unwanted, potentially immunogenic, contaminants into the bulk API. If the fouling is severe, it may block the column, increase back pressure and reduce flow rate. Regular CIP prevents the build up of these contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of CaptivA® resin.

## CIP Protocols

*Note: The following CIP protocols are intended to* serve as *a guideline only. Cleaning protocols specific for a given feed material should be developed and optimized by the end user.*

Typically, CIP is conducted every 5 cycles but this will ultimately depend on the nature of the feed material. Different contaminants require different or even combine CIP protocols. Severe fouling will require specific protocol development.

*NOTE: 0.1 N or greater NaOH solutions will reduce the resin capacity over time therefore its* use *should be limited to final cleaning prior to column storage or for sanitization prior to the start of a campaign.*

1. Precipitated or denatured substances:
   - Wash with 2 column volumes of one of the following:
     - 50 mM NaOH with or without 1.0 M NaCl
     - M $H_3PO_4$, with or without 1.0 M NaCl
     - 50 mM NaOH in 1.0 M $Na_2SO_4$
     - 0.1-2 M Acetic Acid, 20% EtOH
   - Wash immediately with at least 5 column volumes of 0.2 µm filtered binding buffer at pH 7-9
   - Reverse flow direction.

2. Hydrophobically bound substances
   - Wash the column with 2 column volumes of a non ionic detergent (e.g. cone. 0.1-1% of Tween or Triton® X-100).
   - Wash immediately with at least 5 column volumes of
   - sterile filtered binding buffer at pH 7-9. Reverse flow direction.

OR

   - Wash the columns with 3-4 columns volumes of 70% ethanol or 30% isopropanol.

*Note: Specific regulations may apply when using high concentrations of alcohol since it can require the use of explosion proof areas and equipment.*

- Wash immediately with at least 5 columns volumes of sterile filtered binding buffer at pH 7-9.
- Reverse flow direction.
- Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

*Notes:*

1. *Apply for an approximate contact time of 10 minutes on the column.*
2. *Apply for an approximate contact time of 30 minutes on the column.*
3. *Apply for an approximate time of 16 minutes on the column.*

### Sanitization

Sanitization protocols are used to reduce microbial contamination of the resin bed, often prior to storage. Effective sanitization prevents the build up of microorganisms that can lead to endotoxin contamination or a fouled resin bed.

*Note: Exposure times require end user validation to confirm effective sanitization /microbial kill*

There are common approaches to sanitization protocols such as:

1. Equilibrate the column with a solution of 0.1 M acetic acid and 18.5% ethanol OR 2% benzyl alcohol in 0.1 M acetic acid. Allow to stand for 1 hour, and then wash with at least 5 column volumes of sterile binding buffer

2. Equilibrate the column with 70% ethanol Allow to stand for 12 hours, and then wash with at least 5 column volumes of sterile binding buffer.

*Note: Specific regulations may apply when using 70% ethanol since it can require the use of explosion proof areas and equipment.*

3. Flow 0.1 N NaOH over column for 30 minutes (approx 30- 100 cm/hr). Immediately equilibrate resin to —pH 7.5 with 5-7 times the column volume of isotonic buffer that has been sterile filtered (2uM).1x PBS or 10x PBS is most common, but other isotonic buffers are acceptable. Prior to storing resin, rinse with 5-7 times the column volume with sterile water to remove all NaOH and buffer salts.

*NOTE: 0.1 Nor greater NaOH solutions will reduce the resin capacity over time therefore its use should be limited to final cleaning prior to column storage or for sanitization prior to the start of a campaign.*

### Storage

OPUS® columns should be equilibrated in buffer containing a preservative such as 2/3 PBS, 18.5% ethanol to prevent microbial growth. Ensure that the column plugs are fully tightened and store at +2 to +8 °C. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

## Order Information

OPUS® pre-packed columns are designed for flexibility such that the columns can be custom made for bed height and chromatography media. The following table describes the current range and availability. Please call Repligen to discuss your specific needs.

| Cat Number | Resin/Volume | Column Style/ø | Bed Height |
|---|---|---|---|
| BS-012-CPRI-010<br>BS-012-CPRI-020 | CaptivA® resin ~11 mL<br>CaptivA® resin ~ 23 mL | OPUS® 1.2 cm<br>OPUS® 1.2 cm | 10 cm<br>20 cm |
| | | | |
| BS-025-CPRI-010<br>BS-025-CPRI-020 | CaptivA® resin ~49 mL<br>CaptivA® resin ~98 mL | OPUS® 2.5 cm<br>OPUS® 2.5 cm | 10 cm<br>20 cm |
| | | | |

For more information or questions, please contact Repligen:

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