CaptivA® HF
Protein A Affinity Resin

User Guide

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INSPIRING ADVANCES IN BIOPROCESSING

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Abbreviations

A_s	Peak asymmetry
API	Active pharmaceutical ingredient
CF	Compression factor
CIP	Clean-in-Place
CV	Column volume
DBC	Dynamic binding capacity
EQ	Equilibration
GMP	Good manufacturing practice
HCP	Host cell protein
HETP	Height equivalent to a theoretical plate
HF	High Flow
mAbs	Monoclonal antibodies
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PBS	Phosphate buffered saline
PPE	Personal protective equipment
rSPA	Recombinant Protein A
RT	Residence time

1. Introduction

CaptivA® HF Affinity Resin is a cross-linked agarose based Protein A affinity resin designed for bind and elute capture process chromatography for the purification of Fc-fusion proteins and monoclonal antibodies (mAbs). CaptivA® HF Protein A Affinity Resin is designed to have similar performance characteristics to CaptivA® PriMAB Protein A Affinity Resin (manufactured by Repligen) with improved flow rate capability. The resin is constructed using components and protein immobilization chemistry commonly used for the commercial manufacture of biologics (see Section 4 for more information).

The CaptivA® HF Affinity Resin is available in loose resin formats and in OPUS® Pre-packed Chromatography Columns for rapid implementation.

This user guide provides general guidance for the use of CaptivA® HF Affinity Resin. For further optimization or troubleshooting support, please contact your local Repligen Field Application Scientist (FAS). If you need assistance contacting your local FAS, the Customer Service team at Repligen would be happy to help (email: customerserviceUS@repligen.com; phone: 781-250-0111).

2. About this document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

3. Safety precautions

Table 2. Safety precautions for CaptivA® HF Affinity Resin

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	This product is shipped in an 18.0 ±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.
WARNING 	Flammable liquid and vapor. • Keep away from heat/spark/open flame/hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/ international regulations.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

4. Product description

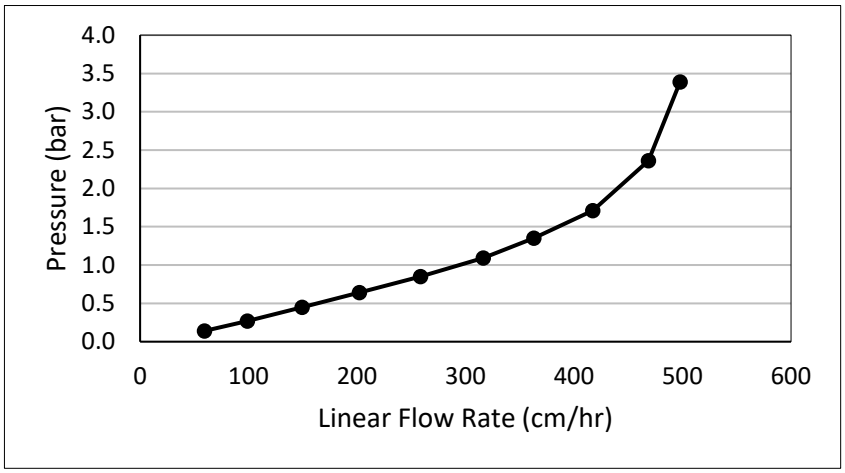
CaptivA® HF Affinity Resin comprises an engineered affinity ligand immobilized to a highly cross-linked agarose support matrix. The CaptivA® HF Affinity Resin enables a high purity capture step which decreases process time and improves overall yield in the production of mAbs and Fc-fusion molecules.

Table 3. Product characteristics

Characteristics	Description
Matrix composition	Highly cross-linked agarose
Ligand	Recombinant protein (E. coli expression; (rSPA) Animal Free)
Average particle size	82 µm
Coupling chemistry	Multi-point attachment *via* reductive amination
Maximum flow velocity	350 cm/hr
Operational pressure	DO NOT EXCEED 1.5 bar deltaP
Operating temperature	2 - 30°C Do not freeze
Delivery conditions	Shipped at room temperature 50% slurry in 18% ethanol
Recommended pH:	Operational: 3 - 10 Clean-in-Place (short term): 2 - 13
Storage conditions	18 - 20% ethanol or 2% benzyl alcohol
Storage temperature	2 - 8°C

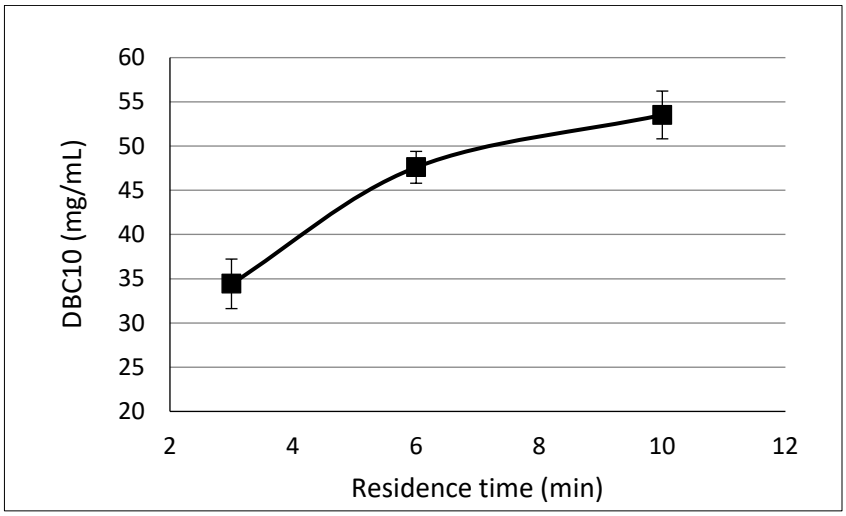
CaptivA® HF Affinity Resin was packed in an OPUS® 45R Column (45 cm ID x 20 cm L). Compression factor: 1.25. Pressure drop was measured with increasing linear flow rate from 50 - 500 cm/hr (Figure 1). The new base bead allows for improved pressure flow properties; linear flow rates over 400 cm/hr can be sustained while pressure remains less than 2 bar.

Figure 1. Pressure-flow properties of CaptivA® HF Affinity Resin



Dynamic binding capacity (DBC) of polyclonal hIgG was calculated at 10% breakthrough (1 mL column; Figure 2). Data was collected at residence times of 3 min, 6 min, and 10 min (n = 4; data presented as mean ± standard deviation). DBC appreciably increased as residence time increased from 3 min - 10 min.

Figure 2. Average DBC vs. residence time for CaptivA® HF Affinity Resin



5. Product specifications

Table 4. Product specifications

Specification	Value
Static binding capacity	≥ 40 mg human IgG/mL resin
Leachable ligand	≤ 10 ng rSPA per mL resin

6. Use recommendations

As with most affinity chromatography media, CaptivA® HF Affinity Resin offers high selectivity for antibodies and Fc-fusion molecules. The combination of high binding capacity and high flow capability affords excellent resin productivity.

The primary aim of optimizing the affinity step (or, optimizing the binding buffer) is to establish the conditions that bind the highest amount of target molecule, in the shortest time, with the highest product recovery. The degree to which Protein A binds to IgG varies with respect to both the origin and antibody subclass. There might even be a substantial diversity in binding characteristics within a single subclass. This is an important consideration when developing the purification protocol.

To enhance the binding strength of the resin and achieve efficient capture of the target antibody, it is often necessary to modify the binding buffer in one of the following ways:

- Increase pH to reduce electrostatic repulsion between Protein A and IgG and allow an uninhibited affinity interaction
- Increase salt concentration to reduce electrostatic repulsion and increase hydrophobic interactions
- Reduce temperature of the feed and column to improve binding

The affinity of Protein A affinity resins like CaptivA® HF Affinity Resin varies for antibodies of different species, classes and subclasses varies; as such, 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. This can be optimized by changing pH and salt conditions.

Table 5. Use recommendations

Use	Recommendation
Flow rate	Loading: ≥ 4 min RT; preferred ≥ 6 min RT to maximize capacity Equilibration (EQ)/ Wash: ≥ 2 min RT Elution/ CIP: ≥ 3 min RT Maximum flow rate (45 cm D X 20 cm L column): 350 cm/hr Operational pressure: DO NOT EXCEED 1.5 bar deltaP
Loading pH	7.2 - 7.8
Equilibration and wash buffer	Phosphate buffered saline (PBS), pH 7.2-7.8 Wash buffer composition and volume may require optimization Additional secondary wash buffers may be used to improve HCP clearance: • PBS, 1 M NaCl • 20 mM sodium citrate, pH 5.5-6.0
Elution buffer	100 mM acetic acid, pH 3.3
Strip	200 mM acetic acid (upflow recommended)
CIP (See Section 6.1)	CIP (upflow recommended) Five (5) cycle cadence example: • Cycle 1 - 4: 50 mM NaOH/1M NaCl, 15 - 30 min contact time • Cycle 5: 0.1 M NaOH, 15 min contact time
Storage solution	18 - 20% ethanol or 1 - 2% benzyl alcohol

RT = residence time

6.1 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 active pharmaceutical ingredient (API). If the fouling is severe, it may block the column, increase back pressure, and reduce flow rate. Regular CIP prevents the buildup of these contaminants in the packed bed, and helps to maintain the capacity, flow properties, and general performance of CaptivA® HF Affinity Resin.

6.1.1 CIP protocols

The following CIP protocols are intended as an initial guideline. Typically, CIP is conducted every 5 cycles or prior to storage; however, the frequency of CIP will ultimately depend on the nature of the feed material. It may be necessary to run more than one CIP protocol if the resin is contaminated with a diverse range of contaminants. Severe fouling will require specific protocol development. Cleaning steps should be performed in upflow direction.

Precipitated or denatured substances:

- Wash with 2 - 5 column volumes of 0.1 M H_3PO_4 or 50 mM NaOH in 1.0 M NaCl
- Wash immediately with at least 5 column volumes of water or equilibration buffer
- Minimum contact time of 15 minutes

Note: *Extended contact time in NaOH will negatively impact binding capacity.*

Hydrophobically bound substances

- Wash the column with 2 column volumes of a nonionic detergent (e.g., conc. 0.1%)
- Wash immediately with at least 5 column volumes of water or equilibration buffer

OR

- Wash the columns with 3 - 4 columns volumes of 70% ethanol or 30% isopropanol
- Wash immediately with at least 5 column volumes of water or equilibration buffer
- Apply increasing concentration gradients to avoid air bubble formation when using high concentrations of organic solvents

Table 6. General protocol

Step	Buffer	Residence time	CV
Equilibration	PBS	≥ 2 min	5
Load	Clarified culture media	≥ 4 min	-
Wash 1	PBS	≥ 2 min	3
Wash 2	PBS, 1 M NaCl	≥ 2 min	5
Wash 3	PBS	≥ 2 min	3
Elution	100 mM acetic acid, pH 3.5	≥ 3 min	3 - 5
Strip	200 mM acetic acid	≥ 3 min	3
CIP cycle 1 - 4	50 mM NaOH, 1 M NaCl	≥ 3 min (15 - 30 min contact)	3
CIP cycle every 5th	0.1 M NaOH	≥ 3 min (15 min contact)	3

*Flow rate limits will depend on column geometry, DO NOT EXCEED 1.5 bar deltaP.

7. Column packing instructions

- The resin is supplied as 50% slurry in 18% ethanol. In preparation of column packing, exchange the shipping solution with 0.1 M NaCl three times. Alternatively, phosphate buffered saline (PBS) can be used as the packing buffer. The resin may be packed with flow pack or axial compression methods.
- Determine slurry concentration by gravity settling or by centrifugation using a 15 mL conical tube, swing bucket rotor at 1800 rpm for 15 minutes. Read immediately.

7.1 Recommended compression factor

- Column ID < 2.5 cm: 1.15 - 1.20
- Column ID > 2.5 cm: 1.20 - 1.25

7.2 Flow packing

1. Decant storage solution and re-suspend resin in the desired packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.15 - 1.25, depending on column diameter and packing pressure).
4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled To Column.
5. Set the three-way valve to the *pump to purge* position and prime the flow path.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the three-way valve purge line until air is purged.

7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump. Close the bottom column port.
8. Set the three-way valve to the *column to purge* position. Manually lower the flow adaptor until it reaches 0.5 - 1 cm above the settled bed. Liquid should purge through the top of the column via the three-way valve.
9. Set the three-way valve to the *pump to column* position. Open the bottom column port and flow buffer up to a pressure of 1.5 bar deltaP and mark the top of the resin bed once stabilized. Maintain flow for a minimum of 3 CV. Stop the pump. Close the bottom column port.
10. Set the three-way valve to the *column to purge* position and manually lower the flow adapter to the bed height marked in the previous step.
11. Set the three-way valve to *pump to column* position. Open the bottom column port. Flow buffer for 3 CV at a flow rate that creates 1.5 bar to condition the column. If a gap forms between the flow adaptor and the bed, lower the adapter and repeat the previous step. Do not flow at the packing pressure again or the column will continue to compress.
12. Flow condition the column.
    a. 2 - 3 CV in down-flow at 300 cm/hr
    b. 2 - 3 CV in up-flow at 300 cm/hr
    c. 2 - 3 CV in down-flow at 300 cm/hr
    d. 2 - 3 CV in up-flow at 300 cm/hr
13. Evaluate column performance using HETP and asymmetry.
    a. (HETP) > 1800 N/m
    b. Peak asymmetry 0.8 - 1.8

Note: *If HETP is < 1800 N/m and asymmetry > 1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and re-test. If asymmetry < 0.8, reduce compression in 0.02 CF increments and re-test.*

7.3 Axial compression

1. Decant storage solution and re-suspend resin in the desired packing buffer. The recommended packing buffer is 0.1 M NaCl. Alternatively, phosphate buffered saline (PBS) or water can be used as the packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.15 - 1.25, depending on column diameter and packing pressure).
4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled To Column.
5. Set three-way valve to the *pump to purge* position and prime the flow path. Close the outlet port of the column and connect the flow adapter to the top of the column.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the top port until air is purged.
7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump.
8. With the pump stopped, keep the three-way valve in the *pump to column* position and the bottom outlet port open, lower the flow adaptor at a rate of 150 cm/hr until the target compression factor is achieved (liquid will flow out of the bottom port of the column).
9. Flow condition the column with an additional 3 CVs packing buffer at a flow rate that achieves 1.5 bar. If a gap forms between the flow distributor and the bed, lower the adapter and repeat flow condition step above. Do not flow at the packing pressure again or the column bed will continue to compress.
10. Flow condition the column.
    a. 2 - 3 CV in down-flow at 300 cm/hr

b. 2 - 3 CV in up-flow at 300 cm/hr
c. 2 -3 CV in down-flow at 300 cm/hr

d. 2-3 CV in up-flow at 300 cm/hr

11. Evaluate column performance using HETP and asymmetry.
    a. (HETP) > 1800 N/m
    b. Peak asymmetry 0.8 - 1.8

Note: *If HETP is < 1800 N/m and asymmetry > 1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and re-test. If asymmetry < 0.8, reduce compression in 0.02 increments and re-test.*

8. Column qualification

Column qualification is typically determined by testing HETP (height equivalent to a theoretical plate) and A_s (peak asymmetry).

IMPORTANT: For best results, avoid sample dilution by applying the sample as close to the column inlet as possible, and placing the conductivity meter as close to the column outlet as possible.

Table 7. Recommended column efficiency testing parameters

Condition	Recommendation
Detection	Conductivity
Effluent solution	0.1 M NaCl
Sample volume	1% of the column volume
Sample concentration	1 M NaCl
Flow rate	100 cm/hr

9. Ordering information

CaptivA® HF Affinity Resin is available in loose resin formats and in OPUS® Pre-packed Chromatography Columns for rapid implementation.

More information regarding OPUS® Pre-packed Chromatography Columns can be found by visiting https://www.repligen.com/technologies/opus.

Table 8. Part numbers for CaptivA® HF Affinity Resin

Resin volume	Part number
5 mL	CA-HF-0005
25 mL	CA-HF-0025
100 mL	CA-HF-0100
1 L	CA-HF-1L

10. Index