CaptivA®
HF Protein A
Affinity Resin

Regulatory Support File

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Abbreviations

AF	animal-free
API	active pharmaceutical ingredient
ASTM	American Society for Testing Materials
BBS	Batch Binding Station
BCMS	Business Continuity Management System
BSE	bovine spongiform encephalopathy
C	Celsius
CFU	colony forming unit
CIP	Clean-in-Place
cm	centimeter
CNC	Controlled Not Classified
CV	column volume
DBC	Dynamic binding capacity
ELISA	enzyme-linked immunosorbent assay
EQ	Equilibration
EtOH	ethanol
EU	endotoxin unit
Fc	Fragment, crystallizable
GMP	Good manufacturing practice
H3PO4	phosphoric acid
HEPA	High efficiency particulate air (filter)
HF	High Flow
hIgG	human immunoglobulin gamma antibody
HPLC	High-performance liquid chromatography
hr	hour
ID	internal diameter
IgG	immunoglobulin gamma antibody
ISO	International Organization for Standardization
L	length

M	Molar
MA	Massachusetts
mAbs	Monoclonal antibodies
mg	milligram
min	minute
mL	milliliter
mM	millimolar
n	number
N	normal
N/A	not applicable
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
nm	nanometer
OEM	original equipment manufacturer
OPUS	Open Platform User Specified
P/N	part number
PBS	Phosphate buffered saline
PM	preventative maintenance
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
QC	Quality Control
QMS	Quality Management System
RODI	reverse osmosis/deionization
rSPA	native recombinant Staphylococcal Protein A
rSPA	Recombinant Protein A
SEC	size-exclusion chromatography
SVHC	substances of very high concern
T	time
T0	time: zero
TSA	tryptic soy agar
TSE	transmissible spongiform encephalopathy
USP	US Pharmacopeia
UV	ultraviolet
ΔP	delta pressure
µg	microgram
µm	micrometer (micron)
µS	microSeimen

1. Introduction

This Regulatory Support File presents product information for CaptivA® HF Protein A Affinity Resin from Repligen Corporation. As an engineer, scientist, or manufacturer, you may need this information to guide your validation activities, including process development, writing validation protocols, and scaling up.

If you are new to chromatography, reading this validation guide will help you learn about CaptivA HF Protein A Affinity Resin and the procedures required to use it successfully.

When you purchase CaptivA HF Protein A Affinity Resin, you receive user instructions as well as product quality and performance data. This product quality and performance data, combined with the information in this support file (and the data collected from your process) provide much of the information you need to validate your process in an effective and efficient manner.

2. Quality documentation

2.1 Quality policy

2.1.1 Repligen Corporation – A higher standard

Repligen Corporation has over 50 years of experience providing products that meet the quality required in bioprocessing applications. We can satisfy the quality needs of customers with particular application requirements. Full compliance with regulatory requirements and meeting customer needs are the driving forces for Repligen higher standard of quality.

2.1.2 Complying with quality regulations

Since product quality is essential to our customers' success, Repligen makes quality assurance a top priority. Repligen is an ISO 9001 certified company and has an established (QMS) Quality Management System.

ISO statement: Product is manufactured in compliance with Repligen ISO 9001 certified Quality Management System. Our ISO certification certificate can be found on the Repligen website.

2.1.3 Product certification

Repligen supplies certificates of analysis for released manufacturing lots. Upon request, you can receive a certificate of analysis for an individual lot of CaptivA HF Protein A Affinity Resin. Certificates of analysis are substantiated with data appropriate to each product.

Substantiation data is maintained on file for 5 years or as required by customers and OEM applications.

2.1.4 Animal Free and BSE/TSE Free statement

Repligen manufactures CaptivA HF Protein A Affinity Resin in the United States of America using materials sourced from qualified suppliers. No material of animal origin is used in the raw materials or manufacturing process for this product, and therefore CaptivA HF Protein A Affinity Resin is free of transmissible spongiform encephalopathy (TSE) and bovine spongiform encephalopathy (BSE).

3. Product description

This chapter describes the design and performance of CaptivA HF Protein A Affinity Resin.

3.1 Product design

CaptivA HF Protein A Affinity resin is constructed of commonly used components including cross-linked agarose beads, recombinant Protein A and immobilization chemistry which individually have been used in the commercial manufacture of many therapeutic biologics. CaptivA HF Resin is designed for bind and elute capture process chromatography for the purification of Fc-fusion proteins and monoclonal antibodies (mAbs).

This resin product has utility as a purification tool in the manufacturing of both therapeutic and diagnostic proteins and is applicable at laboratory discovery, process development, clinical and commercial manufacturing scale for processes producing a few milligrams to tens of kilograms of protein.

CaptivA HF Resin is a modern style affinity resin combining high static binding capacity and flow properties with low Protein A leakage. The CaptivA HF Resin performance profile, in combination with lower cost of ownership, has enabled Repligen to create a Protein A resin that has a compelling economic value proposition that makes it suitable for processes where high performance, high quality and economics are the key drivers:

1. Modern processes where higher flow rates are desired.
2. Processes that can be cleaned with lower (< 0.2 M) levels of caustic.
3. Processes requiring fewer numbers of cycles.
4. Disposable manufacturing processes where single-use or campaigned columns are used and require a cost-effective disposable resin.

CaptivA HF Protein A Affinity resin uses rSPA (native recombinant *Staphylococcal* Protein A) affinity ligand, a recombinant Protein A produced in *Escherichia coli* by Repligen. rSPA is an exact amino acid for amino acid copy of the native Protein A extracted from *Staphylococcus aureus*. rSPA is manufactured by recombinant expression in a very high titer *E. coli* fermentation process. rSPA is made in a soy/yeast extract-based fermentation and, as such, is recognized as animal-free (AF). rSPA provides similar binding specificity to the Fc region of IgG as both the original rProtein A and native *Staphyloccocal aureus* Protein A, providing excellent purification in one step.

Repligen designed rSPA to be an identical and functional version of the native Protein A molecule (Table 1).

Native Protein A consists of three different regions (Figure 1):

1. Signal Sequence
2. IgG Binding Domains
3. C-terminal X Domain

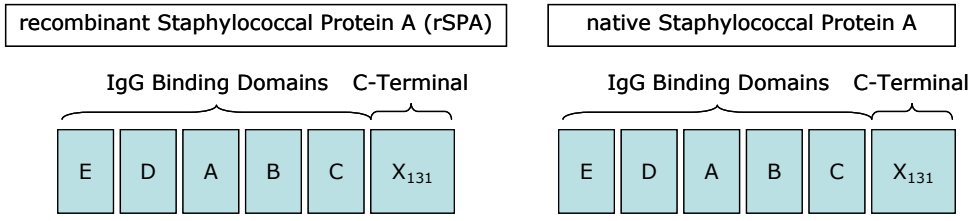
The signal sequence is responsible for directing the protein to the correct location in vivo. The five IgG binding domains (E, D, A, B, C) are homologous functional binding regions. The C-terminal X domain is divided into Xc and Xr regions which are thought to be responsible for attachment of Protein A to the bacterial cell wall.

Table 1. Characteristics of Native vs Recombinant Protein A

Characteristic	Repligen rSPA	native Protein A Protein A	Repligen rPA50/srPA50
Molecular Weight	46.7 kDa	46.7 kDa	44.6 kDa
IgG Binding - E, D, A, B, & C Regions	Yes	Yes	Yes
hIgG Binding	>95%	>95%	>95%

Note: *rPA50 contains 18 amino acids of the beta-glucuronidase (BG) protein derived from the gene promoter system at its N terminus and 91 amino acids of the X domain at its C-terminus. Native Protein A contains 131 amino acids of the X domain.*

Figure 1. Protein A functional structure



3.2 Materials of construction

- Highly cross-linked agarose beads with a particle size of 45 – 165 µm (Cross-linked using Epichlorohydrin chemistry)
- rSPA - Recombinant Native Protein A (Repligen), >95% pure. Manufactured by chromatographic and ultra-filtration purification of a genetically modified E. coli fermentation lysate. The ligand is immobilized onto the base resin by reductive amination.
- 18.5% Ethanol preservative (removed during column packing)
- Purified water
- Protein A is immobilized using reductive amination coupling chemistry. This coupling method ensures low ligand leakage (≤5 ng rPA/mL). The protein A ligand itself is produced in genetically modified E. coli using a soy-based media. rSPA is designated animal-free (AF).

Repligen rSPA QC release testing confirms that critical quality attributes are met. Historical test method requirements for rProtein A products were compared to the Repligen internal test methods that are used during release testing (Table 2). Repligen has identified three test methods that are performed differently than the test methods described in the current USP.

Table 2. Test methods for rProtein A products and Repligen rSPA method comparison

Analysis	Repligen Method
Bioburden	Membrane filtration followed by TSA plate culture
Endotoxin	Commercially available chromogenic endotoxin kit
Total protein	UV absorbance at 275 nm
Identity by SDS-PAGE	2 µg load onto 10% Bis-Tris SDS-PAGE stained with Coomassie R-350
Purity	HPLC by SEC: Dilute to 1 mg/mL, absorbance at 214 nm and 280 nm
Identity by hIgG binding	Binding by HPLC IgG Column at 280 nm
UV Spectra	% transmittance at 400 – 500 nm

3.3 Technical specifications

Table 3. CaptivA HF Protein A Affinity Resin properties

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 ΔP
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

3.4 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 maintain the capacity, flow properties, and general performance of CaptivA HF Affinity Resin.

3.4.1 CIP protocols

The following CIP protocols are intended as an initial guideline. Typically, CIP is conducted every five (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:

1. Wash with 2 – 5 column volumes of 0.1 M H3PO4 or 50 mM NaOH in 1.0 M NaCl for a minimum contact time of 15 minutes.
2. Wash immediately with at least 5 column volumes of water or equilibration buffer.

Note: Extended contact time in NaOH will negatively impact binding capacity, resulting in loss of performance.

Hydrophobically bound substances

1. Wash the column with 2 column volumes of a nonionic detergent
2. Wash immediately with at least 5 column volumes of water or equilibration buffer

OR

1. Wash the column with 3 – 4 column volumes of 70% ethanol or 30% isopropanol
2. Wash immediately with at least 5 column volumes of water or equilibration buffer

Note: *Apply increasing concentration gradients to avoid air bubble formation when using high concentrations of organic solvents.*

Table 4. General protocol

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

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

3.5 Performance qualification

3.5.1 Static Binding Capacity

The static capacity of CaptivA HF Resin is determined based on its ability to bind and elute a solution of human polyclonal IgG (hIgG) based on a standard protocol.

1. Remove ethanol from the gel by rinsing.
2. Mix the gel with hIgG solution.
3. Incubate at room temperature (pH 7.4) for 30 minutes.
4. Remove unbound protein by multiple washes in PBS (4 washes total).
5. Elute bound IgG with 0.2 M glycine buffer (pH 2.0) and quantify by UV absorbance.
6. The release specification is ≥40 mg/mL.

This specification is supported by data from the process development scale-up and process manufacturing validation batches (Table 5).

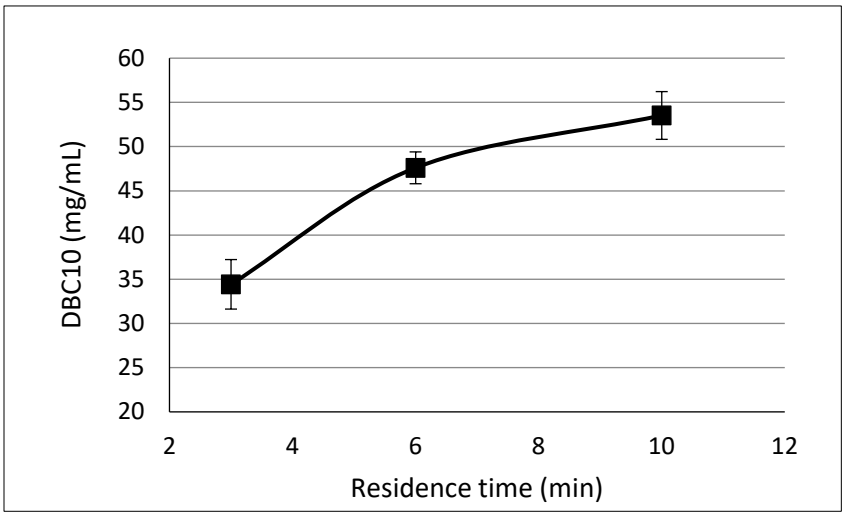
Table 5. CaptivA HF Resin Binding Capacity Lot Release Data

CaptivA HF Resin Lot Number	hIgG binding mg/mL
IP191202	50
IP191430	51
IP191279	47
IP191278	50

3.5.2 Dynamic Binding Capacity (DBC)

Dynamic binding of antibody to CaptivA HF Resin may be end-user specific, thus determination must be made on a process/product-specific basis. For informational purposes, dynamic binding was determined using human polyclonal immunoglobulin as the reference antibody.

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

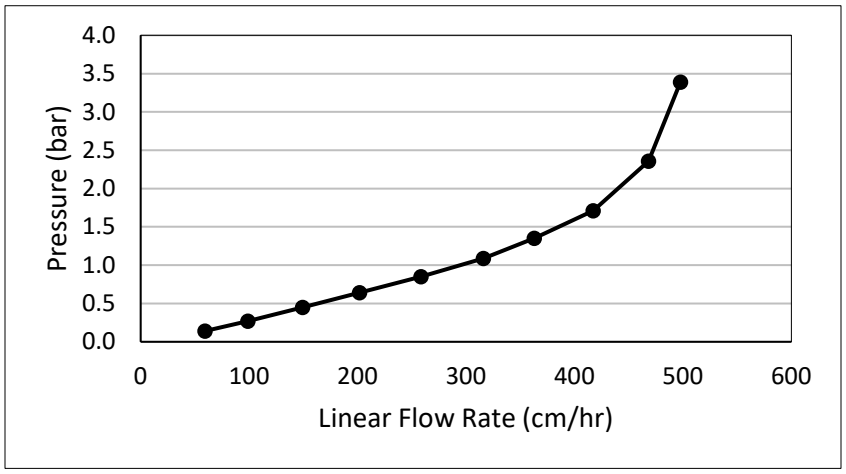


Dynamic binding capacity of polyclonal hIgG was calculated at 10% breakthrough (1 mL column; Figure 2). Data was collected at residence times of 3, 6, and 10 minutes (n = 4; data presented as mean ± standard deviation). DBC appreciably increased as residence time increased from three to 10 minutes.

3.5.3 CaptivA HF Resin pressure flow characteristics

The recommended maximum flow velocity for CaptivA HF Resin is 350 cm/hr or pressure <1.5 bar. The column diameter will significantly impact the maximum flow potential of the resin. A smaller diameter column will typically achieve a greater maximum flow rate.

Figure 3. Pressure flow curve



CaptivA HF Affinity Resin was packed in an OPUS® 45R Column (45 cm ID x 20 cm L) using a compression factor of 1.25. Pressure drop was measured with increasing linear flow rate from 50 – 500 cm/hr (Figure 3). The base bead allows linear flow rates of over 400 cm/hr to be sustained while pressure remains less than 2 bar.

3.5.4 CaptivA HF Resin CIP Stability

CaptivA HF Resin may be cleaned and sanitized with a weak NaOH solution resulting in a moderate decrease in resin capacity over extended exposure times (Table 6).

Table 6. DBC Data and Percent DBC Remaining of T0 after 0.1 N NaOH Exposure

0.1 N NaOH Exposure (Hours)	6 minutes DBC (mg/mL resin)	Percent DBC Remaining
0	47.9	100.0
1	47.5	99.2
2	46.8	97.7
3	45.8	95.6
5	44.4	92.7
8	42.0	87.7
10	40.6	84.8
15	38.1	79.5
20	36.4	76.0
25	35.0	73.1

3.5.5 Protein A Leaching

The Protein A leaching assay is designed to quantify the amount of Protein A that may be released by the resin during a typical elution process. Using a standard protocol:

1. Elution is simulated using 0.1 M sodium citrate buffer at pH 3.0.
2. The simulated elution is collected and analyzed for Protein A using Protein A ELISA assay (commercially available as Repligen catalog number 9000-1).

This process is supported by data from the process development scale-up and process manufacturing validation batches (Table 8).

Table 7. CaptivA HF Resin Leached Protein A Lot Release Data

CaptivA HF Resin Lot Number	rProtein A Leakage ng rPA/mL
IP191202	3
IP191430	3
IP191279	4
IP191278	2

4. Biocompatibility

4.1 Extractables and leachables

Repligen has not tested the CaptivA HF Protein A Affinity Resin for extractables.

Note: *The materials of construction of Captiva HF Protein A Affinity Resin have been used in products manufactured and marketed by Repligen for several years. Affinity resins using these materials have been validated for use in the manufacture of regulated therapeutics approved for human use.*

For CaptivA HF Protein A Affinity Resin, Repligen recommends that customers undertake characterization studies under their specific process conditions. The following list of chemicals are used during manufacture of CaptivA HF Protein A Affinity Resin:

- Highly cross-linked agarose
- Hydrochloric acid
- Sodium metaperiodate
- Sodium hydroxide
- Sodium carbonate
- Sodium chloride
- Sodium borohydride
- Recombinant native Protein A
- Ethanol

4.2 REACH assessment

The CaptivA HF Protein A Affinity Resin is comprised of two components: the affinity ligand and an agarose resin bead. The affinity ligand is exempted from the registration requirements due to production volume not exceeding one metric ton. The resin bead is exempt from registration due to its polymer classification. The vendor (Agarose Beat Technologies) has indicated that the REACH legislation is not applicable to the bead.

The dry weight of CaptivA HF Protein A Affinity Resin imported into the European Economic Area is not projected to exceed one metric ton annually, so the final resin product is also exempt from registration. Both REACH statements also identify that no substances of very high concern (SVHC) are used or that SVHC are below the legal limits and compliant with the Candidate List of SVHC. The REACH compliance assessment is ongoing with Assent Compliance, however, at this time, no obligations for product registration or reporting of SVHC have been identified.

5. Packaging, storage and labeling

5.1 Packaging

CaptivA HF Protein A Affinity Resin is packaged in bottles of 5 mL, 25 mL, 100 mL and 1 L. CaptivA HF Protein A Affinity Resin is also available in OPUS Pre-packed Chromatography Columns.

5.2 Shipping

CaptivA HF Protein A Affinity Resin is stored at 2 – 8°C and shipped at ambient temperature in standard/existing bottle packaging consistent with other similar affinity resin products. Fill sizes up to 1 L are packaged in appropriate size polyethylene terephthalate glycol (PETG) bottles. Fill sizes greater than 1 L are packaged in high density polyethylene (HDPE) bottles.

5.3 Storage and shelf life

CaptivA HF Protein A Affinity Resin is designed for multi-campaign use over a period of months or years. Since the launch of CaptivA HF Protein A Affinity Resin in 2019, Repligen has a 37-month time point for long-term shelf life stability showing capacity and leaching results meeting specifications (Table 8). Additionally, an accelerated stability study was performed for 14 days at 37°C and shows static capacity and leaching results meeting specifications (Table 9). Proper storage at 2 – 8°C in a bacteriostatic preservative like 18 – 20% EtOH or 2% benzyl alcohol must be adhered to in order to maximize shelf life.

Note: *Cell culture processes, chromatography methods, and cleaning protocols will influence the lifetime of the resin in a process. Justification for using CaptivA HF Protein A Affinity Resin in a manufacturing process can also be built using a downscale cycle performance model. This process specific data can also be used to risk-assess the long-term use of CaptivA HF Protein A Affinity Resin.*

Table 8. 37-month stability testing of CaptivA HF Protein A Affinity Resin

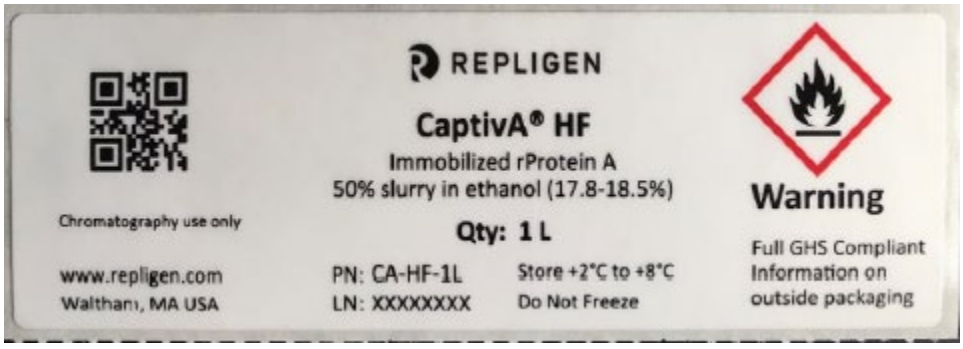
CaptivA HF Resin Lot Number	Static Binding (hIgG mg/mL) Specification: ≥40	Leached Protein A (ng/mL) Specification: ≤10
IP191430	50	2
IP191279	50	4
IP191202	50	2

Table 9. Accelerated Stability (37°C for 14 days) of CaptivA HF Protein A Affinity Resin

	Static Binding Capacity (hIgG mg/mL) Specification: ≥40	
Lot Number	T = 0 days	T = 14 days
IP191202	50	53
IP191279	47	52
IP191430	51	52
	Leached rSPA Ligand IgG (ng/mL) Specification: ≤ 10	
Lot Number	T = 0 days	T = 14 days
IP191202	3	2
IP191279	4	1
IP191430	3	3

5.4 Labeling and product identification

Figure 4. Product label



5.4.1 Traceability and bar codes

Procedures and methods are established to identify the product and status of product throughout Repligen manufacturing process and distribution. Our unique lot number identification allows us to trace production and material details of any product.

6. Manufacturing information

6.1 Manufacturing introduction

Immobilized resin manufacturing is performed at Repligen corporate headquarters: 41 Seyon Street, Waltham, Massachusetts, 02453, USA. Products manufactured in this facility do not require registration or market approval. Neither the facility nor products manufactured herein are subject to regulatory review or audit. CaptivA HF Protein A Affinity Resin consists of the Repligen

recombinant ligand covalently linked to highly cross-linked agarose formulated to approximately 50% suspension in 18% ethanol as a preservative.

6.2 Manufacturing business continuity

Repligen recognizes the importance of continuity of supply for these critical purification products. Repligen also recognizes the need for a pragmatic use of dual sourcing for critical manufacturing raw materials. Repligen maintains a risk-based Business Continuity Management System (BCMS) for all bioprocessing products. The aim of the BCMS is to ensure a reliable and uninterrupted supply of product to key customers in the event of any incident that might disrupt normal business operations. Therefore, Repligen has taken steps to identify and mitigate against business risks in the manufacturing of bioprocessing products.

The BCMS maintained by Repligen recognizes that dual sourcing is not always the answer. In many cases there is no equivalent product or, if there is, managing complex validation matrices and meaningful supply volumes can create other problems. Repligen, through a product-by-product approach, utilizes a combination of validated second sourcing where practicable and carefully planned raw material and finished goods inventory in tandem with a second facility manufacturing rebuild plan. The result is manageable inventories that can cover the necessary time required to restart and revalidate manufacturing. Furthermore, for customers with supply agreements, Repligen will maintain a minimum inventory level at a remote storage facility.

6.3 Manufacturing facilities

Bioprocessing of this affinity resin occurs at two facilities:

- Repligen Waltham, MA (USA): rSPA protein ligand manufacturing and immobilization
- Agarose Bead Technologies (Madrid, Spain): Bead manufacturing

6.3.1 Fermentation

Encompassing raw material storage, media preparation, strain handling and main fermentation, this area is used for large scale recombinant E. coli fermentation.

6.3.2 Recovery

Encompassing product recovery, intermediate purification laboratory, and intermediate storage freezer, this area is used for recovery and buffer exchange of the ligand prior to final purification.

6.3.3 Controlled Not Classified (CNC) Area

The CNC area is a strictly controlled and monitored area used for final purification and immobilization and fill/finish of the ligand. All rooms are on a cleaning and disinfection schedule.

Access is restricted to authorized personnel only. Gowning procedures are strictly observed. Environmental monitoring is performed to check for viable contamination.

The design of the Repligen manufacturing facility allows effective segregation of manufacturing processes and dedicated/disposable equipment is used wherever possible. Processes that require shared equipment have rigorous area batch clearance protocols to prevent cross contamination.

6.3.4 Shipping

Finished product is stored in monitored temperature-controlled units in a facility that is physically separate from the manufacturing site.

6.4 Manufacturing control

Training: Manufacturing is performed by qualified and trained operators. Training documentation is maintained by Document Control.

Process documentation: Repligen manufacturing processes are governed by an ISO 9001 compliant Quality System. All manufacturing work instructions are contained in controlled documents and are issued in advance of each manufacturing batch. Batches and sub-batches are 100% traceable through an internal lot numbering system. All manufacturing data are recorded by operators at the time of manufacturing.

Raw materials: All raw materials and suppliers are controlled. Each raw material has a pre-approved specification, and every receipt of material is reviewed prior to use in manufacturing.

Process change control: Manufacturing process changes are governed by Repligen change management procedures.

Product storage control: Product is stored in temperature-controlled units. All units have chart recorders and alarms that are constantly monitored.

Calibration control: Equipment and monitoring devices are controlled through the Repligen Equipment Control process. Each piece of equipment is uniquely identified and has a preventative maintenance (PM) and/or calibration schedule as necessary.

High purity water: Purified water is supplied to all manufacturing areas from a reverse osmosis/deionization (RODI) plant. The RODI system is fully automated and provides high quality water in a continuously circulating loop. The water system at Repligen has been designed to provide water quality that meets specifications, making it fit for purpose (Table 11). Water quality is routinely monitored by Repligen Quality Control.

Table 10. Repligen water specifications vs. ASTM, USP purified, and WFI

Assay	ASTM type I	USP purified water	WFI	Repligen specification
Conductivity	≤0.056 µS/cm	≤1.3 µS/cm	≤1.3 µS/cm	≤0.01 mS/cm
LAL	≤0.03 EU/ml	≤0.25 EU/ml	<0.25 EU/mL	≤0.5 EU/mL
Bioburden	≤ 10 CFU/1000 ml	≤100 CFU/mL	≤0.1 CFU/mL	≤10 CFU/mL
pH	N/A	5 – 7	5 – 7	5 – 7
TOC	≤50 ppb	≤0.5 ppm	≤0.5 ppm	≤0.1 ppm

Repligen has set these specifications in conjunction with routine maintenance that ensures that the quality of the water produced by the system.

6.5 Manufacturing CaptivA HF Protein A Affinity Resin

The Protein A ligand is produced by fermentation of a recombinant E. coli. After the protein is recovered from the fermentation broth, the protein is purified to ≥95% purity by a series of filtration and chromatography steps. The immobilization process is performed in the clean room, using a Batch Binding Station (BBS). The BBS serves as a mixing vessel for the immobilization chemistry. The frit and fluid outlet/drain valve allow the manufacturing operators to drain liquid from the vessel while retaining the resin within the unit.
Cross-linked agarose is obtained from a third-party supplier. The agarose is transferred to the BBS and washed with RODI water to remove storage buffer and preservative. The rSPA ligand is coupled to the resin via a proprietary reductive amination process. Following the immobilization phase, the resin is transferred to the final suspension buffer, 18% ethanol, as a bacteriostatic agent. The slurry is adjusted to contain approximately 50% solids.
After the slurry has been volume-adjusted and measured, the finished product is bottled and labeled. The fill/finish operation is carried out under HEPA filtered air. The final product is stored at 2 – 8°C.

6.6 QC lot release testing

Upon completion of manufacturing, the product is placed into 2 – 8°C storage, and samples (taken during fill/finish) are submitted to QC for release testing:

- Reconciliation and inspection: Physical count to verify quantities, and inspection of container/label integrity
- Appearance: Visual inspection to ensure conformity of appearance (white to off-white slurry, ~50% fill, no foreign particulates)
- Determination of CaptivA HF Resin fill: A sample of the product is centrifuged to separate the solid and supernatant phases. Percent fill is determined volumetrically.
- Quantification of Protein A leaching: Determination of the quantity of Protein A that will leach under typical elution conditions. The amount of leaked rProtein A that would contaminate an antibody preparation purified using CaptivA HF Resin is measured by ELISA. The specification is ≤10 ng rProteinA /mL.
- Determination of rProtein A hIgG binding: The static binding capacity is determined using human polyclonal IgG. The specification is >40 mg of hIgG per mL of resin.Decant storage solution and re-suspend resin in the desired packing buffer.
- Attach bottom flow adaptor to column body.

Table 11. Affinity ligand quality specifications

Test/Characteristic	Specification
Appearance (liquid)	Clear, pale yellow with no particulates
Bioburden	≤5 CFU/mL
Endotoxin	≤1.0 EU/mg
Protein concentration	50 mg/mL ± 10%
10% SDS-PAGE Coomassie Stain	Single major band, ~47,000 Daltons
Purity, HPLC	≥98% at 214 nm ≥95% at 280 nm
hIgG Binding	≥95%
Conductivity	≤0.1 mS/cm
UV Spectrum (400 – 500 nm)	>80% Transmittance

Table 12. Resin quality Specifications

Test/Characteristic	Specification
Visual inspection	White to off-white slurry with no foreign particulates
Static binding capacity	≥40 mg hIgG/ml
Ligand leaching	≤10 ng rProteinA/ml
Bioburden	≤50 CFU/ml

6.7 Resin Release Assays

A summary of the assays and release specifications for CaptivA HF Protein A Affinity Resin is given below.

6.7.1 Determination of sample appearance by visual inspection (QCP-1127)

The bottled affinity resin is inspected visually for color and presence of foreign materials. The specification is white to off-white slurried media with no foreign particulates.

6.7.2 Determination of resin binding capacity (QCP-1161)

The static capacity is determined based on its ability to bind and elute a solution of hIgG. The hIgG solution is incubated with the immobilized base matrix at room temperature (pH 7.4). Unbound protein is removed by multiple washes in PBS. The bound hIgG is then eluted with 0.2 M glycine buffer (pH 2.0) and quantified by UV absorbance. Release specification for static binding capacity is ≥40 mg hIgG per mL of resin.

6.7.3 Quantitation of hIgG protein ligand leaching (QCP-1042)

The amount of leaked affinity ligand that would contaminate a product elution pool purified using CaptivA HF Protein A Affinity Resin is measured by ELISA (P/N: 9000-1). The result is reported as nanogram affinity ligand per milliliter of elution supernatant.

6.7.4 Determination of bioburden (QCP-10074 or M-QCP-1003)

Two (2) mL of sample is individually applied to 0.2 µm cellulose nitrate membrane filters. Each of the filters is then rinsed with 100 mL of sterile peptone water prior to being transferred to a tryptic soy agar (TSA) plate. Following five days of incubation at 30 – 35°C, the plate is examined for the growth of colonies. The assay is accepted if there is no visible growth on the negative control plate. The release specification for bioburden content is ≤50 CFU/mL.

7. 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 www.repligen.com/technologies/opus.

Table 13. 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

8. Index

Customer Service

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