Native Recombinant Staphylococcal Protein A Ligand (rSPA)

REGULATORY SUPPORT FILE

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

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1 Introduction

The Regulatory Support File for the Repligen rSPA (native recombinant Staphylococcal Protein A) Affinity Ligand is intended to be used as:

- a guide for appropriate application use in process development, clinical and commercial purification processes
- a guide to validation in manufacturing processes
- a support reference for CMC submissions for regulatory license approval
- a guide for supplier audits
- in place of a Drug Master File (DMF) submission. Repligen offers end users open access to the critical product quality and manufacturing information in this Regulatory Support File in lieu of limited access afforded by the DMF system.

Repligen is committed to providing all relevant technical, manufacturing and quality information, however, only non-confidential information is presented in this document. Confidential details may be made available upon request through a formal confidentiality agreement or as part of a supplier audit.

Quality Policy

Copies of the Repligen quality policy, and ISO certificate can be found on http://www.repligen.com.

Safety Notices

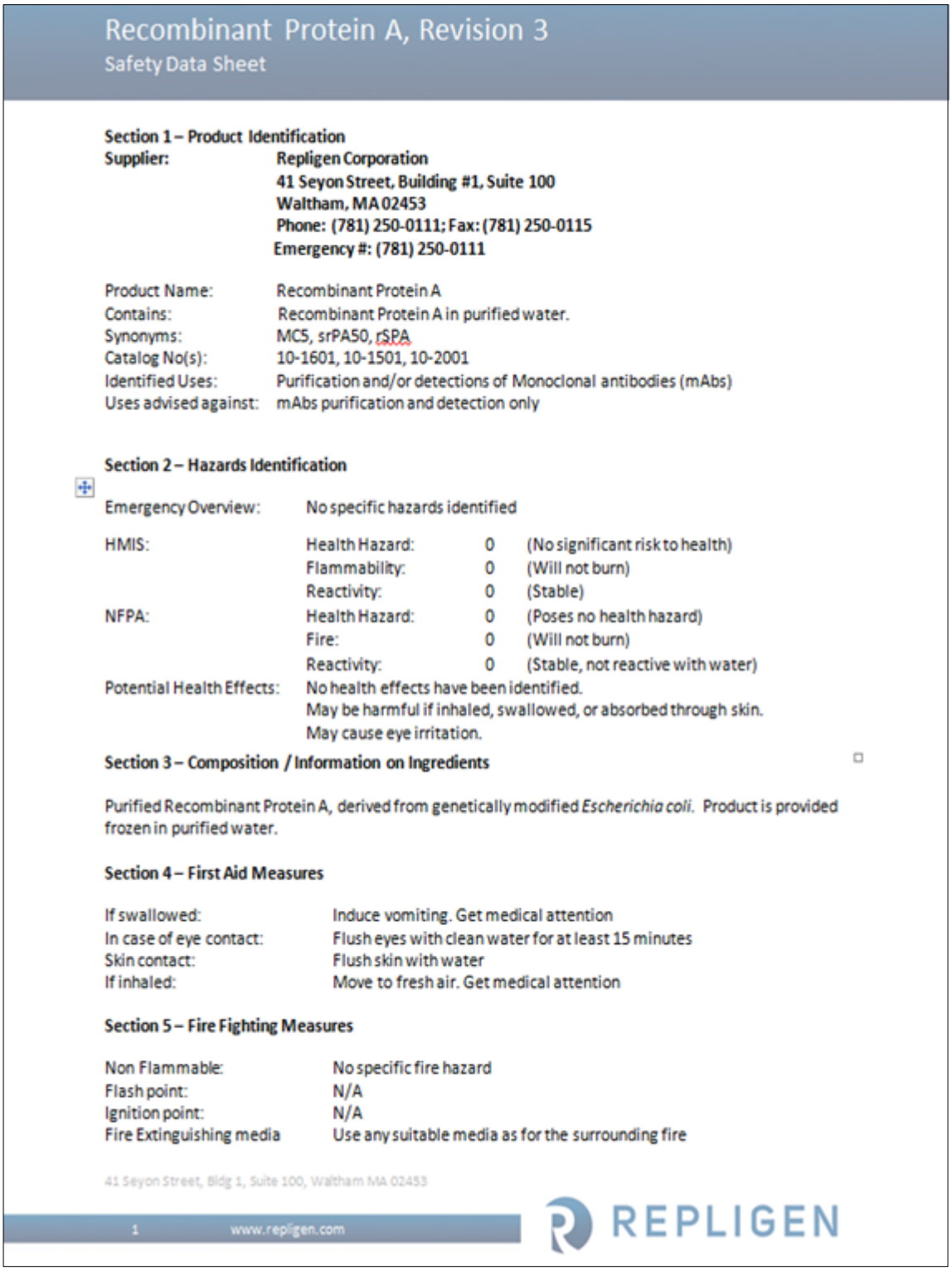
- Follow all local regulations for safe disposal
- For laboratory and manufacturing production use only
- Not for administration to humans
- Reference SDS for product-specific safety information

Responsible Official

The individual below is designated responsible for quality and regulatory affairs for Repligen Corporation. All correspondence or requests for audits should be addressed to:

Senior Director of Quality
Claire McGrath
Tel: +1-781.250.0111
Email: cMcgrath@Repligen.com

Figure 1. Safety Data Sheet



Recombinant Protein A, Revision 3

Safety Data Sheet

Section 1 – Product Identification

Supplier: **Repligen Corporation**
41 Seyon Street, Building #1, Suite 100
Waltham, MA 02453
Phone: (781) 250-0111; Fax: (781) 250-0115
Emergency #: (781) 250-0111

Product Name:	Recombinant Protein A
Contains:	Recombinant Protein A in purified water.
Synonyms:	MC5, srPA50, rSPA
Catalog No(s):	10-1601, 10-1501, 10-2001
Identified Uses:	Purification and/or detections of Monoclonal antibodies (mAbs)
Uses advised against:	mAbs purification and detection only

Section 2 – Hazards Identification

Emergency Overview:	No specific hazards identified		
HMIS:	Health Hazard:	0	(No significant risk to health)
	Flammability:	0	(Will not burn)
	Reactivity:	0	(Stable)
NFPA:	Health Hazard:	0	(Poses no health hazard)
	Fire:	0	(Will not burn)
	Reactivity:	0	(Stable, not reactive with water)
Potential Health Effects:	No health effects have been identified.		
	May be harmful if inhaled, swallowed, or absorbed through skin.		
	May cause eye irritation.		

Section 3 – Composition / Information on Ingredients

Purified Recombinant Protein A, derived from genetically modified *Escherichia coli*. Product is provided frozen in purified water.

Section 4 – First Aid Measures

If swallowed:	Induce vomiting. Get medical attention
In case of eye contact:	Flush eyes with clean water for at least 15 minutes
Skin contact:	Flush skin with water
If inhaled:	Move to fresh air. Get medical attention

Section 5 – Fire Fighting Measures

Non Flammable:	No specific fire hazard
Flash point:	N/A
Ignition point:	N/A
Fire Extinguishing media	Use any suitable media as for the surrounding fire

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Recombinant Protein A, Revision 3

Safety Data Sheet

Section 6 – Steps to be Taken in the Event of a Spill or Discharge:

Personal Protection:	Wear lab coat, gloves and eye protection. Treat with procedures appropriate for biological materials. Soak up spill with absorbent material and collect in a closed container suitable for incineration. Clean the affected area with disinfectant solution. Do not allow material to enter soil, waterways or drains.
Disposal procedure:	Dispose of in accordance with all applicable federal, state, and local environmental regulations.

Section 7 – Handling and Storage

Ventilation:	Keep in a well ventilated area
Respiratory Protection:	N/A
Eye/skin Protection:	Standard laboratory practices recommended.
Storage:	Keep container closed. Store frozen for optimum shelf life.
Special precautions:	N/A

Section 8 – Exposure Controls/Personal Protection

General:	Standard laboratory practices recommended. Clean any exposed skin after handling, before leaving the working area, and before eating, smoking or using the lavatory. Dispose of, or clean any contaminated clothing before re-use.
PPE:	Personal protective equipment should be selected to provide adequate protection based upon the procedures being performed. Wear laboratory coat, gloves and safety glasses when handling. Respiratory protection not required

Section 9 – Physical and Chemical Properties

Appearance:	Frozen aqueous solution
pH:	pH 5 - 8
Flash point:	Will not burn
Ignition point:	Will not ignite
Explosion limits:	No risk of explosion
Solubility:	Soluble in water

Section 10 – Stability and Reactivity

Stability:	Stable
Hazardous polymerization:	Will not occur
Decomposition products:	No known hazardous decomposition products

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Section 11 – Toxicological Information

Acute toxicity:	No known significant effects
Irritation:	No known significant effects. May be a skin or eye irritant.
Sensitization:	No known significant effects
Carcinogenicity:	No known significant effects
Mutagenicity:	No known significant effects
Teratogenicity:	No known significant effects

Section 12 – Ecological Information

No known hazards

Section 13 – Disposal Considerations

Dispose of in accordance with all applicable federal, state, and local environmental regulations.
Do not allow spilled material to enter soil, waterways or drains

Section 14 – Transport Information

IATA:	Not classified
DOT Road Transport:	Not Regulated

Section 15 – Regulatory Information

OSHA/SARA/CWA/CAA: No known hazards

EU Risk and Safety Statements:

- Not classified

Section 16 – Other Information

The material published in this Safety Data Sheet has been compiled from our experience and data presented in various technical publications. It is the user's responsibility to determine the suitability of this information for the adoption of necessary safety precautions.

Repligen makes no warranty or representation about the accuracy or completeness nor fitness for purpose of the information contained herein.

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2 Product Information

2.1 Description

Repligen rSPA Affinity Ligand (rSPA) is a recombinant Protein A ligand produced in Escherichia coli (*E.coli*).

- rSPA is an exact amino acid for amino acid copy of the native Protein A extracted from Staphylococcus aureus (Bibliography reference 1 and 2)
- 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 Staphyloccocus 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. Characteristics of Native vs. Recombinant Protein A

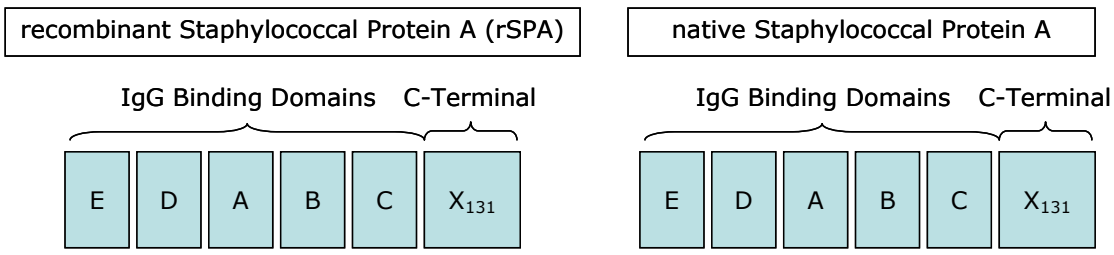
	Repligen rSPA	Native Protein A	Repligen srPA50
Molecular Weight	46.7 kDa	46.7 kDa	44.6 kDa
IgG Binding - E,D,A,B, and C Regions	Yes	Yes	Yes
hIgG Binding	>95%	>95%	>95%

Native Protein A consists of three different regions (Figure 2):

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.

Figure 2. Protein A Functional Structure



2.2 Materials of Construction

rSPA (Recombinant Native Staphylococcal Protein A), is >95% pure. It is manufactured by chromatographic and ultra-filtration purification of a genetically modified *E.coli* fermentation lysate.

- Repligen rSPA QC release testing satisfies the required product quality information outlined in the USP (ref 3) 31 General Chapter <130> for rProtein A
- Purified water

2.3 Technical Specifications

Test Method	Specification
Appearance (liquid)	Clear, pale yellow with no particulates
Bioburden	≤ 5 CFU/mL
Endotoxin	≤ 1.0 EU/mg
Protein Concentration (A_{275})	50 mg/ml ± 10%
12% SDS-PAGE Coomasie Stain	Single major band, ~ 47, 000 Daltons
Purity, HPLC	≥ 98% at 214nm ≥ 95% at 280 nm
hIgG Binding	≥ 95%
Conductivity	≤ 0.1 mS/cm
UV Spectrum (400 – 500nm)	> 80% Transmittance

2.4 Performance Qualification

Performance qualification, against a specification set during process development, has been established by demonstrating reproducibility of multiple five (5) lots.

Appearance

	Lot Number	PP101454	PP101453	RN101467	RN100900	RN100919
Assay	**Specification**					
Physical Inspection	**Clear, liquid Pale Yellow No particulates**	Pass	Pass	Pass	Pass	Pass
UV Spectral Analysis	**>80% transmittance**	100.0%	100.0%	100.1%	96.6%	99.7%

Purity and Identity

	Lot Number	PP101454	PP101453	RN101467	RN100900	RN100919
Assay	**Specification**					
Identity by SDS-PAGE	**Major band @ ~47 kDa**	47.3 kDa	47.2 kDa	47.6 kDa	47.8 kDa	47.8kDa
Purity by GPC	**>98% @ 214 nm >95% @ 280 nm**	100% 100%	100% 100%	100% 100%	†99.5%	†99.5%

Note: Data for lot numbers RN100900 and RN100919 represent test methodology prior to the adoption of USP General Chapter methods for GPC purity.

Concentration and Conductivity

	Lot Number	PP101454	PP101453	RN101467	RN100900	RN100919
Assay	**Specification**					
Concentration A_{275}	**50mg/mL ± 10%**	52.6 mg/mL	53.2 mg/mL	51.3 mg/mL	50.9 mg/mL	51.4 mg/mL
Conductivity	**≤ 0.1 mS/cm**	0.0193 mS/cm	0.0199 mS/cm	0.0193 mS/cm	0.0218 mS/cm	0.0232 mS/cm

For optimum shelf life, Repligen recommends that rSPA should be stored frozen at -20±10°C. However, short-term studies suggest that the protein may be stored in closed containers for short periods at room temperature. Care should be taken to avoid microbial contamination during handling.

Binding Capacity

	Lot Number	PP101454	PP101453	RN101467	RN100900	RN100919
Assay	**Specification**					
hIgG Capacity	**≥95%**	99.8%	99.8%	99.8%	99.9%	100.1%

Microbiology

	Lot Number	PP101454	PP101453	RN101467	RN100900	RN100919
Assay	**Specification**					
Bioburden	**≤5 CFU/mL**	0	0	0	0	0
Endotoxin	**≤1.0 EU/mg**	<0.5 EU/mg	<0.5 EU/mg	<0.5EU/mg	<0.1EU/mg	<0.1EU/mg

rSPA has been shown by Repligen to be stable for 48 months (Table 2).
Additional studies have shown that:

1. There is no significant change in rSPA purity, potency or hIgC binding activity following up to three freeze-thaw cycles.
2. The rSPA product shows no significant change in purity or hIgG binding after 14 days at 37°C.
3. The rSPA product shows no significant change in purity or hIgG binding after 5 days of vigorous shaking at 37°C or 7 days at ambient temperature.

Table 2. rSPA Stability Data

rSPA Ligand IgG Binding %

Lot Number	Time Point	Time Point	Original Specification
	(0 months)	(48 months)	
RN092563	100%	99.8%	≥ 95%
RN092600	100%	99.5%	≥ 95%
RN092619	100%	99.6%	≥ 95%

rSPA Ligand Purity by SEC %

Lot Number	Time Point	Time Point	Original Specification
	(0 months)	(48 months)	
RN092563	Test not required at the time †	99.9%	≥ 95% @ 280nm
	99.5%	99.4%	≥ 98% @ 214nm
RN092600	Test not required at the time †	99.8%	≥ 95% @ 280nm
	99.5%	98.6%	≥ 98% @ 214nm
RN092619	Test not required at the time †	99.8%	≥ 95% @ 280nm
	99.3%	99.1%	≥ 98% @ 214nm

rSPA Ligand Purity by SDS Page (kD)

Lot Number	Time Point	Time Point	Original Specification
	(0 months)	(48 months)	
RN092563	One major band ~47,000 Daltons	One major band ~47,000 Daltons	One major band ~47,000 Daltons
RN092600	One major band ~47,000 Daltons	One major band ~47,000 Daltons	One major band ~47,000 Daltons
RN092619	One major band ~47,000 Daltons	One major band ~47,000 Daltons	One major band ~47,000 Daltons

Note: Data for lot numbers RN092563, RN092600, and RN092619 represent test methodology prior to the adoption of USP General Chapter methods for GPC purity in June 2010.

3 Product Safety

3.1 Toxicity Profile

Recombinant Protein A

No known toxic effects; no records are found on either Toxnet or the PAN (Pesticides Action Network) pesticides database, see attached SDS for more information.

4 Manufacturing Information

4.1 Introduction

Repligen rSPA manufacturing, Quality Control, and Quality Assurance operations are located at Repligen Corporate Headquarters, at 41 Seyon Street suite #100, Waltham, Massachusetts, 02453, USA. Neither this facility nor products manufactured in this facility require registration nor market approval. Neither the facility nor products manufactured herein are subject to regulatory review or regulatory audit.

4.2 Quality Assurance Standards and Policy

Repligen recognizes the need for:

- Reproducible product performance and quality
- A formal ISO certified quality system that emphasizes process control, traceability, and product conformance
- A quality system that is continually updated and improved in response to customer feedback
- A quality system that is open and auditable
- Accreditation to a recognized quality standard

The Repligen Quality Policy reflects these needs and the firm commitment to meet or exceed customer expectations. This commitment to customer satisfaction is achieved through:

- A clear focus on customer needs, product quality, on time delivery and customer service
- The establishment and maintenance of a Quality Management System including quality policies, objectives and metrics that meet Repligen organizational and business goals
- The personal commitment of our employees to customer satisfaction and fulfillment of their company responsibilities
- Management's commitment to excellence through continuous review and improvement in our policies, objectives, processes, products, services and business activities

Repligen has established, documented, implemented, and maintains a Quality Management System (QMS) which supports the requirements of ISO 9001, Repligen business goals, and is consistent with bioprocess customers' needs.

The Repligen Quality System is currently certified by BSI America to ISO 9001:2015 (see certification below.)

Figure 3: Certificate of Registration

bsi.

By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 9001:2015

This is to certify that:

RepliGen Corporation
41 Seyon Street
Waltham
Massachusetts
02453
USA

Holds Certificate No: **FM 535355**

and operates a Quality Management System which complies with the requirements of ISO 9001:2015 for the following scope:

The design, development and manufacture of bioprocessing products and provision of services to customers worldwide.

For and on behalf of BSI:

Carlos Pitanga, SVP, System Certification and Compliance

Original Registration Date: 2008-12-18
Latest Revision Date: 2017-12-12

Effective Date: 2017-12-18
Expiry Date: 2020-12-17

Page: 1 of 2

...making excellence a habit.

This certificate remains the property of BSI and shall be returned immediately upon request.
An electronic certificate can be authenticated online. Printed copies can be validated at www.bsigroup.com/ClientDirectory
To be read in conjunction with the scope above or the attached appendix.
Information and Contact: BSI, Kitemark Court, Davy Avenue, Knowlhill, Milton Keynes MK5 8PP. Tel: + 44 345 080 9000
BSI Assurance UK Limited, registered in England under number 7805321 at 389 Chiswick High Road, London W4 4AL, UK.
A Member of the BSI Group of Companies.

Note: A current certificate of Registration is on file at Repligen, a current copy can be obtained by contacting Repligen customer service.

4.3 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 its 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.

BCMS recognizes that dual sourcing is not always the answer. In many cases, there is no equivalent product or if there is then 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 end 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.

4.4 Facilities

The Repligen bioprocessing manufacturing facility consists of 3 main areas.

4.4.1 Fermentation

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

4.4.2 Recovery

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

4.4.3 Controlled Not Classified Area

The CNC area is a controlled area, used for final purification and immobilization and fill/finish of Protein A. The environment is strictly controlled and monitored. Air quality is maintained by 100% HEPA filtered air, which is tested to ISO class 7 for non-viable particulates. 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.

4.4.4 Contract Fermentation

Repligen occasionally uses qualified contractors for certain fermentation operations. In any situation where contractors are used to produce raw material their processes, product and quality systems are audited and support Repligen product and quality standards. Maintaining a secondary fermentation site is part of the Repligen formal BCMS strategy.

4.4.5 Shipping

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

4.5 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 the 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 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. Repligen's water system has been designed to provide water quality such as to make it "fit for purpose". The water system design performance specifications are listed in Table 3. Water quality is routinely monitored by Repligen Quality Control.

Table 3. Repligen Water Specifications Compared with ASTM, USP Purified and WFI

	ASTM Type I	USP Purified water	WFI	Repligen Specification
LAL	≤0.056 µS/cm	≤1.3µS/cm	≤1.3µS/cm	≤0.01 mS/cm
Bioburden	≤0.03 EU/ml	≤0.25 EU/ml	< 0.25 EU/mL	≤0.5 EU/mL
pH	≤10cfu/1000ml	≤100 cfu/mL	≤0.1 cfu/mL	≤10 cfu/mL
TOC	N/A	5-7	5 – 7	5 – 7
Conductivity	≤50ppb	≤0.5 ppm	≤0.5 ppm	≤0.1 ppm

Repligen has set these specifications in conjunction with routine maintenance that ensures that the trend performance of the water system remains within specification. The water system performance to specification trending data for 11 months January to November 2009 show that the water quality is under control and meets Repligen specifications, and exceeds the USP purified water specification, (Table 4).

Table 4. Repligen Water System Quality Performance Data

	Conductivity	Ph	Bioburden	Endotoxin
Spec	<0.01 mS/cm	5-7	10 cfu/mL	0.5 EU/mL
Min	0.001	5.1	0	0.02
Max	0.008	6.94	10	0.443
Mean	0.001	5.553	0.677	0.027
n	154	154	154	154

4.6 rSPA Manufacturing

The rSPA 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.

4.7 rSPA Manufacturing - QC Lot Release Testing

Upon completion of manufacturing, the product is placed into storage at -18°C and samples (taken during fill/finish are submitted to QC for release testing.

The rSPA release tests include:

- **Reconciliation and Inspection:** Physical count to verify quantities and inspection of container/label integrity
- **Appearance:** This is measured by both visual inspection and UV transmittance @400-500nm to ensure compliance with product specifications. Both are results are reported on the product certificate of analysis. This test is not specified by USP 31<130> rProtein A General Chapter Test Methods for rProtein A products.
- **Microbiology:** Both bioburden and endotoxin are measured according to validated USP methods ensuring compliance with both product specifications and USP 31<130> rProtein A General Chapter Test Methods for rProtein A products. Results are reported on the product certificate of analysis.
- **Protein Concentration:** This is measured by UV275 absorbance to ensure compliance with product specification and is reported on the product certificate of analysis. This test is not specified by USP 31<130> rProtein A General Chapter Test Methods for rProtein A products.
- **Identity:** This is measured by SDS page/Coomassie in order to ensure compliance with both product specification and USP 31<130> rProtein A General Chapter Test Methods for rProtein A products. Results are reported on the product certificate of analysis.
- **Purity:** This is measured by HPLC in order to ensure compliance with both product specification and USP 31<130> rProtein A General Chapter Test Methods for rProtein A products. Results are reported on the product certificate of analysis
- **Activity:** This is measured by HPLC IgG column which confirms activity and identity ensuring compliance both product specification and USP 31<130> rProtein A General Chapter Test Methods for rProtein A products. Results are reported on the product certificate of analysis.
- **Conductivity:** This is measured by conductivity meter to ensure compliance with product specification and is reported on the product certificate of analysis. This test is not specified by USP 31<130> rProtein A General Chapter Test Methods for rProtein A products.

Repligen's rSPA QC release testing satisfies the required product quality information outlined in the USP (ref 3) 31 General Chapter <130> for rProtein A.

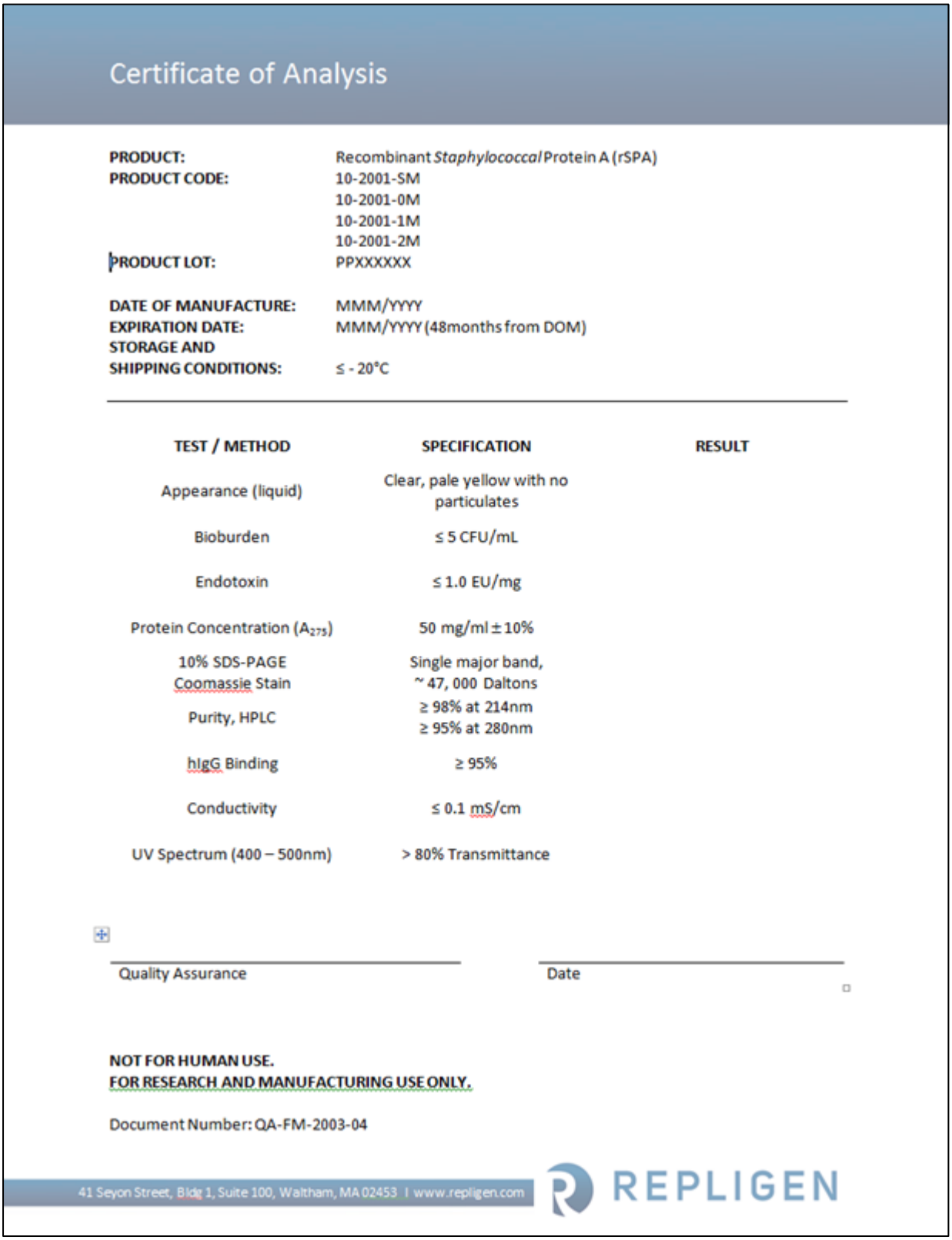
Table 5 outlines the test method requirements of the USP 31 General Chapter <130> for rProtein A which are used during release testing of the rSPA product to achieve the product quality information.

Table 5. USP 31<130> rProtein A General Chapter Test Methods for rProtein A products and Repligen rSPA Method Comparison

Required Analysis	USP General Chapter Method
Bioburden	Parameters contained in general chapter <61>
Endotoxin	Parameters contained in general chapter <85>
Total Protein	Parameters contained in general chapter <851>, dilute to 3 mg/mL, absorbance at 275 nm
Identity by SDS-Page	2µg load onto 10% Bi-Tris stained in Coomassie R-250
Purity	HPLC by SEC: Dilute to 1mg/mL, absorbance at 214nm and 280 nm, L33 packing
Identity by hIgG Binding	Binding by HPLC IgG Column at 280nm
UV Spectral	Not defined in General Chapter

4.8 rSPA Sample Certificate of Analysis

Figure 4: Certificate of Analysis



Certificate of Analysis

PRODUCT:	Recombinant *Staphylococcal* Protein A (rSPA)
PRODUCT CODE:	10-2001-SM 10-2001-0M 10-2001-1M 10-2001-2M
PRODUCT LOT:	PPXXXXXX
DATE OF MANUFACTURE:	MMM/YYYY
EXPIRATION DATE:	MMM/YYYY (48months from DOM)
STORAGE AND SHIPPING CONDITIONS:	≤ - 20°C

TEST / METHOD	SPECIFICATION	RESULT
Appearance (liquid)	Clear, pale yellow with no particulates	
Bioburden	≤ 5 CFU/mL	
Endotoxin	≤ 1.0 EU/mg	
Protein Concentration (A_{275})	50 mg/ml ± 10%	
10% SDS-PAGE Coomassie Stain	Single major band, ~ 47, 000 Daltons	
Purity, HPLC	≥ 98% at 214nm ≥ 95% at 280nm	
hIgG Binding	≥ 95%	
Conductivity	≤ 0.1 mS/cm	
UV Spectrum (400 – 500nm)	> 80% Transmittance	

Quality Assurance _____ Date _____

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5 User Instructions

Specificity and affinity

The degree to which Protein A binds to IgG varies with respect to both the origin and antibody subclass (5).

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 achieve efficient capture of the target antibody it is often necessary to enhance the binding strength by formulation of the binding buffer in one of the following ways.

- By increasing pH, which reduces electrostatic repulsion between Protein A and IgG, allowing an uninhibited affinity interaction
- By increasing salt concentration to reduce electrostatic repulsion and increase hydrophobic interactions
- By reducing the temperature to improve binding

6 Bibliography

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   May 01, 2018
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