mC5 - Recombinant Protein A Ligand

Regulatory Support File

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

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Abbreviations

CMC	Chemistry, manufacturing, and control
Fc region	Fragment crystallizable region
GPC	Gel Permeation Chromatography
HEPA	High Efficiency Particulate Air
HIgG	Human immunoglobulin
HPLC	High-performance liquid chromatography
IgG	Immunoglobulin G
USP	US Pharmacopeia

1. Introduction

The Regulatory Support File for mC5 (recombinant Protein A) Affinity Ligand (Figure 1) from Repligen 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
- 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.

Figure 1. mC5 - Recombinant Protein A

1.1 Quality Policy

Copies of the Repligen Quality Policy and ISO Certificate can be found on www.repligen.com/resources/qualitydocumentation.

1.2 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

1.3 Responsible official

The position 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
Tel: +1-781.250.0111
Email: customerserviceus@repligen.com

1.4 Safety Datasheet

Download the latest mC5 Protein A Ligand Safety Data Sheet from www.repligen.com.

2. Product information

Repligen mC5 Affinity Ligand is a recombinant Protein A ligand produced in Escherichia coli.

- mC5 is an engineered form of Staphylococcus Protein A containing 5 identical modified C-domains repeats
- mC5 is manufactured by recombinant expression in a very high titer *E.coli* fermentation process
- mC5 is made in a soy/yeast extract-based fermentation and as such is recognized as animal free origin (AFO)
- mC5 provides binding specificity to the Fc region of IgG providing excellent purification in one step

Table 1. Characteristics of Repligen Protein A products

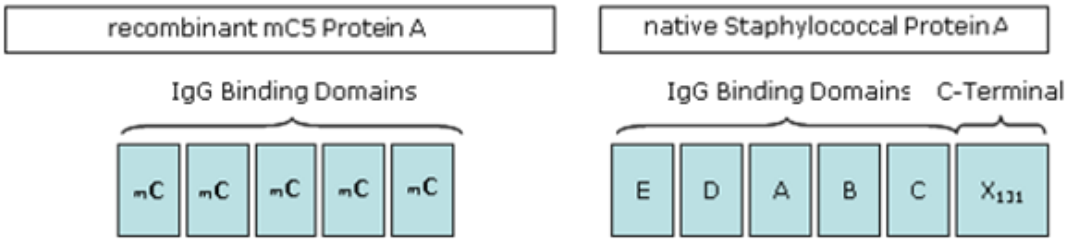
Description	Repligen mC5	Repligen rSPA/Native Protein A	Repligen srPA50
Molecular weight	33.1 kDa	46.7 kDa	44.6 kDa
IgG Binding - E, D, A, B, and C Regions	No – Modified C-Domain	Yes	Yes
hIgG binding	> 95%	> 95%	> 95%

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



2.1 Materials of construction

mC5 (Recombinant Protein A Affinity Ligand), is > 95% pure. It is manufactured by chromatographic and ultra-filtration purification of a genetically modified *E.coli* fermentation lysate.

- Repligen mC5 QC release testing satisfies the required product quality information outlined in the USP (reference 3) 31 General Chapter <130> for rProtein A
- Purified water

1.1 Technical specifications

Table 2. Tests and specifications

Test Method	Specification
Appearance (liquid)	Clear liquid with no particulates
Bioburden	≤ 5 CFU/mL
Endotoxin	≤ 1.0 EU/mg
Protein concentration (A_{276})	45 - 55 mg/mL
SDS-PAGE Coomassie Stain	Single major band, ~ 33 kDa
Purity, HPLC	≥ 95% at 214 nm
hIgG binding	≥ 95%
Conductivity	≤ 0.1 mS/cm
UV spectrum (400 - 500 nm)	> 80% transmittance

2.2 Performance qualification

Performance qualification, against a specification set during process development, has been established by demonstrating reproducibility of multiple three (3) lots.

Table 3. Appearance

Assay	Specification	Lot # JP141496	Lot # JP141511	Lot # JP141521
Physical inspection (liquid)	Clear liquid with no particulates	Pass	Pass	Pass
UV Spectral analysis	> 80% transmittance	96.8%	96.2%	97.0%

Table 4. Purity and identity

Assay	Specification	Lot # JP141496	Lot # JP141511	Lot # JP141521
Identity by SDS-PAGE	Single major band, ~ 33 kDa	Single major band, 32 kDa	Single major band, 32 kDa	Single major band, 32 kDa
Purity by GPC	> 95% @ 214 nm	99.8%	99.6%	99.5%

Table 5. Concentration and conductivity

Assay	Specification	Lot # JP141496	Lot # JP141511	Lot # JP141521
Concentration A_{276}	50 mg/mL ± 10%	49.4 mg/mL	50.2 mg/mL	48.9 mg/mL
Conductivity	≤ 0.1 mS/cm	0.0166 mS/cm	0.017 mS/cm	0.018 mS/cm

For optimum shelf life, Repligen recommends that mC5 should be stored frozen at <-20. However, short-term studies suggest that the protein may be stored in closed containers for up to 7 days at room temperature. Care should be taken to avoid microbial contamination during handling.

Table 6. Binding capacity

Assay	Specification	Lot # JP141496	Lot # JP141511	Lot # JP141521
hIgG capacity	≥ 95.0%	99.8%	99.6%	99.5%

Table 7. Microbiology

Assay	Specification	Lot # JP141496	Lot # JP141511	Lot # JP141521
Bioburden	≤ 5 CFU/mL	0 CFU/mL	0 CFU/mL	0 CFU/mL
Endotoxin	≤ 1.0 EU/mg	< 0.5 EU/mg	< 0.5EU/mg	< 0.5EU/mg

mC5 has been shown by Repligen to be stable for 60 months (Section 2.3). Additional studies have shown that:

1. The mC5 product shows no significant change in purity or hIgG binding after 14 days at 37° C.
2. The mC5 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.

2.3 mC5 stability data

Table 8. mC5 ligand IgG binding %

Lot number	Time point	Time point	Original specification
	(0 months)	(60 months)	
JP141496	98%	99%	≥ 95%
JP141511	97%	100%	≥ 95%
JP141521	99%	100%	≥ 95%

Table 9. mC5 ligand purity by SEC %

Lot number	Time point	Time point	Original specification
	(0 months)	(60 months)	
JP141496	100%	96%	≥ 95% @ 214nm
JP141511	100%	96%	≥ 95% @ 214nm
JP141521	100%	97%	≥ 95% @ 214nm

Table 10. mC5 Ligand purity by SDS page (kD)

Lot #	Time point	Time point	Original specification
	(0 months)	(60 months)	
JP141496	Single major band, ~ 32 kDa	Single major band, ~ 32.3 kDa	Single major band, ~ 33 kDa
JP141511	Single major band, ~ 32 kDa	Single major band, ~ 32.4 kDa	Single major band, ~ 33 kDa
JP141521	Single major band, ~ 32 kDa	Single major band, ~ 32.0 kDa	Single major band, ~ 33 kDa

3. Product safety

3.1 Toxicity profile

3.1.1 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 mC5 manufacturing, Quality Control, and Quality Assurance operations are located at Repligen Corporate Headquarters, at 41 Seyon Street, 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. Download the latest Certificate of Registration from www.repligen.com.

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 2 main areas.

4.4.1 Fermentation

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

4.4.2 Controlled not classified area (CNC)

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 for non-viable and viable particulates. All rooms are on a cleaning and disinfection schedule.

Access is restricted to authorized personnel only. Gowning procedures are strictly followed.

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.

4.4.3 Shipping

Finished product is stored in monitored temperature-controlled units 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, which are issued in advance of each manufacturing batch. Batches and sub batches are 100% traceable through an electronic driven 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 material is quality released prior to use in manufacturing.
- **Process change control:** Manufacturing process changes are governed by the Repligen process and product change management procedures.
- **Product storage control:** Product is stored in temperature-controlled units. All units are monitored and 24/7 alarmed.

- **Calibration control:** Equipment and monitoring devices are controlled through the Repligen Calibration Program. Each piece of equipment is uniquely identified and has a Preventive Maintenance and/or calibration schedule as required.
- **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 Repligen 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 12. Water quality is routinely monitored by Repligen Quality Control.

Table 11. Repligen water specifications compared with USP purified and WFI

Description	USP purified water	WFI	Repligen specification
Endotoxin	≤ 0.25 EU/ml	< 0.25 EU/mL	≤ 0.5 EU/mL
Bioburden	≤ 100 cfu/mL	≤ 0.1 cfu/mL	≤ 10 cfu/mL
pH	5 - 7	5 - 7	5 - 7
TOC	≤ 0.5 ppm	≤ 0.5 ppm	≤ 0.1 ppm
Conductivity	≤ 1.3µS/cm	≤ 1.3µS/cm	< 0.01 mS/cm

Table 12. Repligen water system quality performance data

Description	Conductivity	Ph	Bioburden	Endotoxin
Specification	< 0.01 mS/cm	5 - 7	10 cfu/mL	0.5 EU/mL
Minimum	0.00025	5.16	0	0.053
Maximum	0.00783	6.74	8.5	0.774
Mean	0.00124	5.772	0.262	0.207
n	272	272	272	272

4.6 mC5 manufacturing

The mC5 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 mC5 manufacturing- QC Lot Release Testing

Upon completion of manufacturing, the product is placed into storage at -20° C and samples (taken during fill/finish are submitted to QC for release testing.

The mC5 release tests include:

1. **Reconciliation and inspection:** Physical count to verify quantities and inspection of container/label integrity
2. **Appearance:** This is measured by both visual inspection and UV transmittance @ 400 - 500 nm 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.
3. **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.

4. **Protein concentration:** This is measured by UV276 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.
5. **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.
6. **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.
7. **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.
8. **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 mC5 QC release testing satisfies the required product quality information outlined in the USP (Reference 3) 31 General Chapter <130> for rProtein A.
Table 14 outlines the test method requirements of the USP 31 General Chapter <130> for rProtein A which are used during release testing of the mC5 product to achieve the product quality information.

Table 13. USP 31 <130> rProtein A General Chapter Test Methods for rProtein A products

Required analysis	USP General Chapter Test 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 214 nm and 280 nm, L33 packing
Identity by hIgG binding	Binding by HPLC IgG Column at 28 0nm
UV spectral	Not defined in General Chapter

4.8 mC5 Certificate of Analysis

Request the latest Certificate of Analysis from customerserviceUS@repligen.com

5. User instructions

5.1 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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