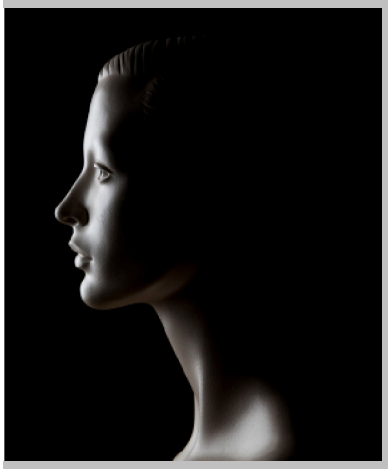
RepliGen BIO PROCESSING

IPA 400HC Protein A Affinity Resin



IPA 400HC Protein A Affinity Resin

REGULATORY SUPPORT FILE

JANUARY 2010

Repligen Corporation
41 Seyon Street
Waltham, MA 02543
USA

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1.0 Introduction

A Regulatory Support File (RSF) is intended to provide information to users of Repligen's products. Specifically this RSF for Repligen's IPA 400HC Immobilized Protein A Affinity resin, is intended to be used as:

a. A guide for appropriate application use in process development, clinical and commercial purification processes.

b. A guide to validation in manufacturing processes

c. A support reference for CMC submissions for regulatory license approval.

d. A guide for supplier audits.

e. A replacement for Drug Master File (DMF) #12874. Repligen has determined that end users would benefit from open access to the critical product quality and manufacturing information in this Regulatory Support File rather than the limited access afforded by the DMF system.

Repligen is committed to providing all relevant technical, manufacturing and quality information. Non-confidential information only is presented in this document. Further, confidential, details will be available either as part of a supplier audit or may be requested and provided under a formal Confidentiality Agreement.

Safety Notices:

- The MSDS advises that this product is shipped in a 0.01% solution of Thimerosal, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use.
- Follow all local regulations for safe disposal
- For laboratory and manufacturing production use only
- Not for administration to humans

Responsible Official;

The individual designated responsible for quality and regulatory affairs for Repligen Bioprocessing, and to whom all correspondence or requests for audits should be addressed:

Deirdre Vaughn – Associate Director of Quality

Tel: (781) 419 0249

Email: dvaughn@repligen.com

2.0 Material Safety Data Sheet

RepliGen

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MATERIAL SAFETY DATA SHEET
Immobilized rProtein A (IPA-400HC) Revision 1 (Effective 01/22/2010)

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

Product Name: ImmobilizedrProteinA (IPA-400HC)
Synonym(s) IPA-400HC
Catalog No(s): 10-2500

SECTION 2 – COMPOSITION / INFORMATION ON INGREDIENTS

Product consists of recombinant Protein A immobilized on cross-linked agarose, prepared as a 50% slurry containing 0.01% thimerosal. Recombinant Protein A is derived from genetically modified *E. coli*.

Substance Name	CAS #	RTECS Number	% by weight	OSHA exposure limit
Thimerosal	54-64-8	OV8400000	0.01%	PEL: 8H TWA 0.01mg/ m^3
Cross Linked Agarose	9012-36-6	-	50%	None known

SECTION 3 – HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW: Poison. Harmful by inhalation and ingestion.
May be harmful through skin contact.

HMIS/NFPA Ratings:

Health Hazard:	**2**	Temporary or minor injury may occur
Fire:	**0**	Will not burn
Instability:	**0**	Normally stable. Not reactive with water

EU Risk phrases: R22: Harmful if swallowed.

Safety Statements: Keep away from food, drink, and animal feeding stuffs. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

SECTION 4 – FIRST AID MEASURES

In case of Ingestion: Wash out mouth with water, provided person is conscious. Consult a physician.

In case of skin contact: Flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Consult a physician.

In case of eye contact: Immediately flush eyes with plenty of water for at least 15 minutes. Assure adequate flushing by separating eyelids with fingers. Consult a physician.

In case of inhalation: Move to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Consult a physician.

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Immobilized Protein A (IPA-400HC)

SECTION 5 – FIRE FIGHTING MEASURES

Suitable media	Dry chemical, CO_2, water spray or foam
Unusual Hazards	Containers may leak if heated. Move containers away from heat or fire (if this can be done without risk)
Special equipment	Wear self contained breathing apparatus for fire fighting if necessary

SECTION 6 – ACCIDENTAL RELEASE MEASURES

In case of leak or spill:	Evacuate area. Allow only suitably trained personnel to proceed with clean up. Provide adequate ventilation. Put on appropriate PPE.
Personal protection (PPE):	Wear safety glasses, lab coat, rubber boots and safety gloves.
Methods for cleaning up:	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform relevant authorities if the product has caused any environmental pollution. Stop leak, if possible without risk. Mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. Do not dispose of in trash or in drains. **Dispose of via a licensed waste disposal contractor.**

SECTION 7 – HANDLING AND STORAGE

Handling:	Wear personal protective equipment (minimum of a lab coat, gloves, and safety glasses). Use in a well ventilated area. Avoid generating splashes or aerosols. Do not get in eyes, on skin or on clothing. Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Avoid prolonged or repeated exposure.
Storage:	Store tightly closed in original container at 2-8 ºC for optimum shelf life
Special precautions:	Keep tightly closed

SECTION 8 – EXPOSURE CONTROLS/PERSONAL PROTECTION

Personal protective equipment:	Should be selected based on the task being performed and the risk involved. Recommended: Wear gloves, laboratory coat and safety glasses when handling. Ensure Safety shower and eye bath are available and operational. Wash contaminated clothing before re-use. Wash skin thoroughly after handling.

Exposure limits

RTECS, Thimerosal:

Source	Type	Value	Remarks
ACGIH	TWA	0.1 mg(Hg)/m^3	Skin
MSHA Std-Air	TWA	0.05 mg(Hg)/ m^3	
OSHA	PEL	8H TWA 0.01 mg(Hg)/ m^3	
NIOSH	Ceiling	0.1mg/ m^3	(SK)

SECTION 9 – PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Colorless liquid containing white suspended solids (50% slurry)
Physical state:	Liquid.
Color:	White solid/colorless liquid
Form:	50% Suspension.
Boiling point:	100ºC

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Immobilized Protein A (IPA-400HC)

SECTION 10 – STABILITY AND REACTIVITY

Stability:	Stable under normal use and storage.
Hazardous polymerization:	Will not occur
Materials to avoid:	No information

SECTION 11 – TOXICOLOGICAL INFORMATION

THIMEROSAL:	
Skin Contact:	May cause skin irritation.
Skin Absorption:	May be toxic if absorbed through skin.
Eye Contact:	May cause eye irritation.
Ingestion:	May be fatal if swallowed. Material may be irritating to mucous membranes and upper respiratory tract.
Sensitization:	Sensitization: Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.
Target organs or systems:	Nerves. Kidneys.
Toxicity data (Thimerosal):	Oral – rat - LD_{50} 75 mg kg^{-1} Subcutaneous – rat - LD_{50} 98 mg kg^{-1} Unreported – rat - LD_{50} 40 mg kg^{-1} IV – mouse - LD_{50} 30 mg kg^{-1} Oral – mouse - LD_{50} 91 mg kg^{-1} Intraperitoneal – mouse - LD_{50} 54 mg kg^{-1} Intra aural – child - LDLO 60 mg kg^{-1}

SECTION 12 – ECOLOGICAL INFORMATION

- No Data

SECTION 13 – DISPOSAL CONSIDERATIONS

- Do not dispose of this product in trash or down drains.
- Contact a licensed professional waste disposal service to dispose of this material.
- Dispose of in accordance with all applicable federal, state, and local environmental regulations.

SECTION 14 – TRANSPORT INFORMATION

- Not regulated

SECTION 15 – REGULATORY INFORMATION

EU Risk phrases	R22: Harmful if swallowed.
Safety Statements:	Keep away from food, drink, and animal feeding stuffs. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/safety data sheets.
US Statements:	Calif. Prop. 65 developmental hazard (Thimerosal)
Target organ(s):	Nerves. Kidneys. Possible sensitizer (Thimerosal)
TSCA inventory item:	Yes (Thimerosal)
California prop – 65	California Prop - 65: This product is or contains chemical(s) known to the state of California to cause developmental toxicity (Thimerosal).

SECTION 16 – OTHER INFORMATION

The material published in this Material 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.

3.0 Product Information

3.1 Product Description

Developed in the Mid 1990's IPA 400HC has passed regulatory scrutiny and is used in the commercial manufacture of therapeutic biologics. IPA 400HC is a cross-linked agarose based Protein A affinity resin designed for bind and elute capture process chromatography in the purification of recombinant proteins and monoclonal antibodies (mAbs).

This affinity 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 mgs to 10's of kilograms of protein.

IPA400HC features low protein leakage and combines good static binding capacity properties with a low cost of ownership that makes it an ideal resin for processes where high quality and economics are key drivers, for example where:

i. Batch volumes are lower and can be easily processed at lower flow rates

ii. Single use columns are used and require a cost effective disposable resin

The affinity ligand "recombinant Protein A" is produced in *Escherichia coli.* This srPA50 ligand is Repligen's "original" recombinant construct "srPA50" derived from the Cowan I strain of *Staphyloccocal aureus*, the lower case *"s"* notation in front of the srPA50, identifies this as a product of a Soy based

fermentation and as such is recognized as AF "ANIMAL FREE". srPA50 provides similar binding specificity to the Fc region of IgG as both rPA50 and native *Staphyloccocal aureus* Protein A, providing excellent purification in one step.

Repligen designed srPA50 to be a functional rather than identical version of the native Protein A molecule, table 1. Native Protein A consists of three different regions, figure 1:

i. Signal Sequence

ii. IgG Binding Domains

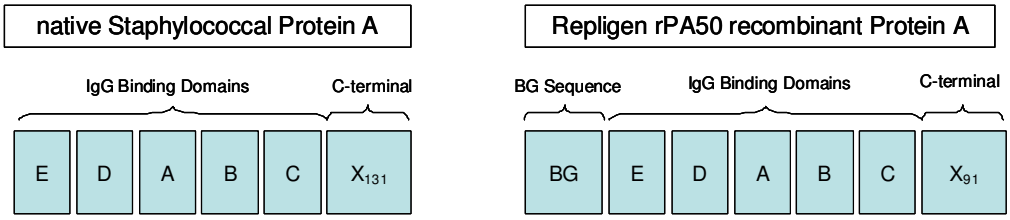
iii. C-terminal X Domain

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. Native Protein A vs. Repligen rPA50 Characteristics

	native Protein A	Repligen rPA50
Molecular Weight	46.7 kDa	44.6 kDa
E,D,A,B,C IgG binding	Yes	Yes
IgG binding	>95%	>95%

Figure 1. Protein A Functional Structure



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.

3.2 Materials of Construction:

- Cross linked 6% agarose beads with a particle size of 45–165 µm (Cross linked using Epichlorohydrin chemistry)
- srPA50 - Recombinant Protein A (Repligen), >98% 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.
- 0.01% Thimerosal preservative (removed during column packing). Purified water.
- Protein A is immobilized using reductive amination coupling chemistry. This coupling method ensures extremely low ligand leakage (< 5ng rPA/mL). The Protein A ligand itself is produced in genetically modified *E. coli*. The lower case *"s"* notation in front of the rPA50, i.e. srPA50 identifies this as an AF "ANIMAL FREE" product identical to rPA50 in every way except that it is manufactured (fermented) in a soy based media.

Repligen's srPA50 is a recognized Protein A affinity ligand as defined by USP 31 General Chapter <130> for rProtein A. Repligen's srPA50 QC release testing satisfies the required product quality information outlined in the USP General Chapter. Table 2 outlines the test method requirements of the USP 31 General Chapter <130> for rProtein A compared to Repligen's internal test methods which are used during release testing of the srPA50 product to achieve the product quality information. Repligen has identified three test methods that are performed differently than the test methods described in the current USP.

Table 2. USP 31<130> rProtein A General Chapter Test Method Comparison

Required Analysis	USP Monograph Method	Repligen Method	Notes
Bioburden	Parameters contained in general chapter <61>	Membrane filtration.	Additional test method required to comply with USP method.
Endotoxin	Parameters contained in general chapter <85>	Commercially available chromogenic endotoxin kit	Aligns with USP 31 <130> test method
Total Protein	Parameters contained in general chapter <851>, dilute to 3 mg/mL, absorbance at	UV absorbance at 275nm	Aligns with USP 31 <130> test method
Identity by SDS Page	rProtein A specific method, 2 µg load onto 10% Bis-Tris stained in coomassie R-250	Tris-Glycine SDS-PAGE stained with Coomassie G	Alteration to test method required to comply with USP method.
Purity	HPLC by SEC: Dilute to 1mg/mL, absorbance at 214 nm and 280nm, L35 packing	HPLC by SEC: absorbance at 214 nm and 280nm	Aligns with USP 31 <130> test method
Identity by hIgG Binding	Binding by HPLC IgG column	Repligen internal method	Alteration to test method required to comply with USP method.
UV Spectral Scan	Dilute sample to 1mg/mL in WFI. Scan at 360-240nm	UV Spectral scan at 360-240nm	Aligns with USP 31 <130> test method
Triton Content	HPLC at 223nm, column packing L11	HPLC at 223nm	Aligns with USP 31 <130> test method
IEF	Dilute sample to 4 mg/mL, run marker set with markers between 3 and 10. Apply 5µL aliquots and run at 1W for 10 minutes, 25W for 40 min, then fix and stain	Commercially available pH 3-10 IEF gel	Aligns with USP 31 <130> test method

Note: At this time Repligen is in the process of updating and transferring its QC methods to ensure compliance with the USP 31 <130> General Chapter.

3.3 Technical Specifications:

Overview – IPA 400HC Properties

Property	Value
Matrix Composition	6% Highly cross linked Agarose
Ligand	Recombinant Protein (srPA50) Animal Free
Particle Size	45 – 165 µm
Coupling Chemistry	Multi-Point Attachment via Reductive Amination
Recommended Working Velocity	30 – 300 cm/hr
Temperature Stability	2 - 40°C Long Term Storage: Store at 2-8°C in a suitable bacteriostatic agent like 20% Ethanol or 0.02% Sodium Azide. **Protect from freezing.**
Delivery Conditions	Shipped RT, 50% Slurry Containing 0.01% Thimerosal
Recommended pH: Working Clean in Place	 3 – 10 2 – 11
Storage Conditions	2-8°C in the presence of a bacteriostatic agent (e.g. 0.01% Thimerosal or 20% Ethanol)
Binding Capacity	Static: 40±3 mg human IgG/ml resin Dynamic: Binding of antibody to IPA 400HC may be end user specific thus determination must be made on a process/product specific basis.
Leachable Protein A	<5ng srPA50 per milliliter resin Protein A leaches from affinity resins as a natural result of protease or caustic degradation of the

Property	Value
	immobilization chemistry or the ligand itself. Leakage of Protein A from IPA 400HC is generally very low due to the application of the multipoint attachment chemistry. Typically, testing with Repligen's commercially available Protein A ELISA Kit (Catalog Number 9000-1) indicates leaching of ≤ 1ng/mL of resin. Despite these very low levels of leached protein A, in most if not all monoclonal applications, it is a requirement that leached Protein A contamination be reduced as far as possible from the final product. This is typically achieved with use of subsequent size exclusion or ion exchange chromatography step and can be monitored using Repligen's Leached Protein A ELISA kits.
Recommended pH working range	3–10 Short term exposure to pH below 3 is sometimes required to elute strongly bound IgG species. However, care must be taken not to denature the protein ligand.
Regeneration	After each separation cycle, regenerate the IPA 400HC resin bed by washing with 3CVs of 0.1M citrate buffer, pH3.

Cleaning-In-Place (CIP) pH 2–11

General recommendations for cleaning IPA 400HC are:

Precipitated or denatured substances: Wash with 2 column volumes of 6 M guanidine hydrochloride 10 mM NaOH, 0.1 M H_3 PO_4 or 50 mM NaOH in 1.0 M NaCl or 50 mM NaOH in 1.0 M Na_2SO_4.

Hydrophobically bound substances: Wash the column with 2 column volumes of a non ionic detergent (0.1%).

The following is a CIP/Sanitization protocol that was adapted for the Immobilized Protein A (IPA) products. This procedure is a guideline only and it is recommended that a protocol be optimized for each specific process. For thorough CIP/sanitization:

1. Flow 0.1-0.2 N NaOH over column for 30 minutes (approx 30-100 cm/hr).
2. Immediately equilibrate resin to ~pH7.5 with 5-7 times the column volume of isotonic buffer that has been 0.2µ filtered. 1xPBS is most common. Other, isotonic buffers are also acceptable.
3. Prior to storing resin, rinse with 5-7 times the column volume with sterile water to remove all NaOH and buffer salts.
4. Store column in 20% Ethanol or 0.02% Sodium Azide at 2 ° to 8° C.
5. Prior to re-using resin, run 5-7 times the cv of sterile filtered buffer through resin to remove residual storage solution.

NOTE: Loss of performance may result from excessive exposure to NaOH.

3.4 Performance Qualification:

Binding Capacity – The specification for IPA 400HC is 40 ± 3mg/mL

The static capacity of IPA 400HC is determined based on its ability to bind and elute a solution of Human polyclonal IgG (hIgG) based on a standard protocol.

1. Remove the Thimerosal preservative from the gel by rinsing with 1x PBS.
2. Mix the gel with the 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. The bound IgG is then eluted with 0.2M Glycine buffer (pH 2.0), and quantified by UV absorbance.
6. The release specification is 40 ± 3mg/mL.

This specification is supported by release data, shown in table 3, from the following 7 lots manufactured between 2004 and 2009:

Table 3. IPA 400HC Binding Capacity Lot Release Data

IPA-400HC Lot Number	Result: Human IgG binding Capacity (mg hIgG/mL)
RN092520	39.2
RN092525	37.2
RN082647	37.5
RN074766	38.0
RN061449	38.8
RN052309	41.6
RN040633	40.7
Mean	39.0
SD	1.6

Dynamic Binding Capacity

Dynamic binding of antibody to IPA 400HC 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.

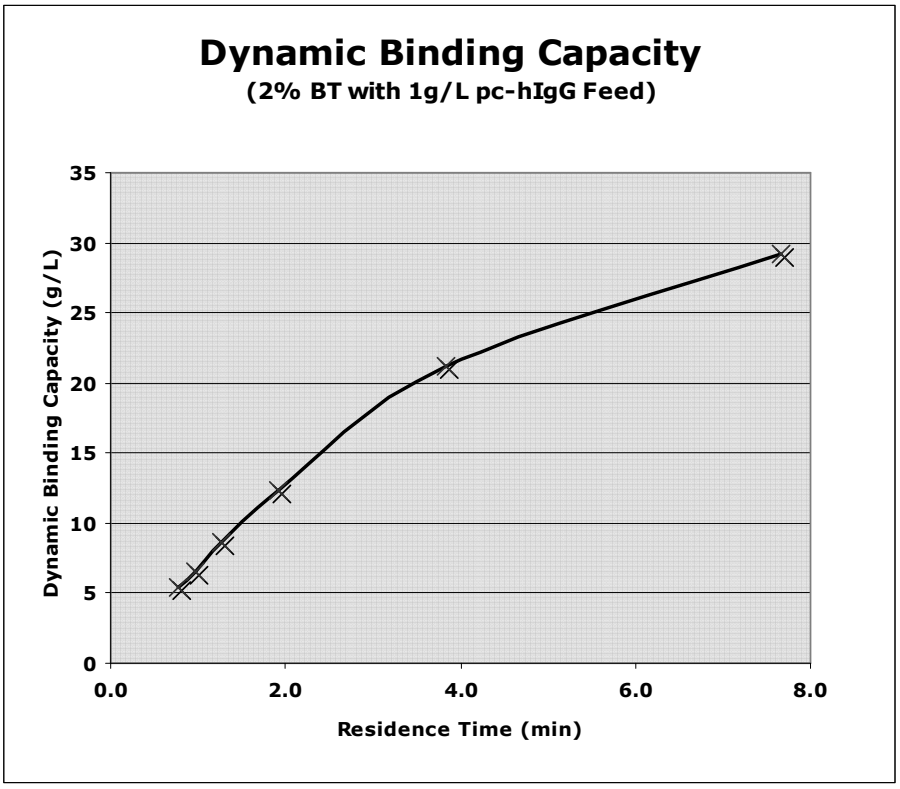
The dynamic binding capacity for IPA 400HC was determined by packing 1 ml of resin into a 0.5 cm diameter column with a final bed height of 5 cm. A 1.0 mg/ml Human polyclonal IgG solution was loaded onto the column at linear flow velocities of 50, 100, 200, 300,400 and 500 cm/hr. Dynamic capacity

was determined at 2% breakthrough after correcting for the unbound IgG3 flow through fraction. The dynamic capacity for IPA 400HC was > 20 g/L at residence times of at least 3 minutes.

Table 4. IPA 400HC Dynamic Binding

Linear Flow [cm/hr]	Residence time [min]	Capacity [g/L]
500	0.8	**5**
400	1.0	**7**
300	1.3	**9**
200	1.9	**12**
100	3.8	**21**
50	7.7	**29**

Figure 2. Graphical Display of IPA 400HC Dynamic Binding



Protein A Leaching - The specification for IPA 400HC is ≤ 5ng/mL.

The Protein A leaching assay is designed to quantify the amount of Protein A that may be released by the IPA 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 eluted protein is collected and analyzed for Protein A using Repligen's fully documented and characterized Protein A ELISA assay (commercially available as catalog number 10-9000-1.

This specification is supported by release data, shown in table 5, from the following 7 lots manufactured between 2004 and 2009:

Table 5. IPA 400HC Leached Protein A Lot Release Data

IPA-400HC Lot Number	Rprotein A Leakage (ng rPA/mL)
RN092520	0.20
RN092525	0.40
RN082647	0.40
RN074766	2.00
RN061449	1.00
RN052309	0.50
RN040633	1.40
Mean	0.84
SD	0.66

IPA 400HC Stability

The IPA 400HC current product stability guideline is 36 months when stored unopened and in compliance with manufacturers recommendations.

Repligen has been making IPA 400HC for over 15 years and all evidence of component stability for the base bead, the srPA50 and immobilized product indicates a shelf life stability of well over three years.

Repligen is currently undertaking a longer-term stability study of IPA 400HC which when complete will be made available to all users.

4.0 Product Safety

4.1 Toxicity Profile

In assembling this toxicity profile Repligen has focused on only the structural components of the resin. The key raw material structural components of IPA 400HC are Agarose and recombinant Protein A.

Thimerosal at 0.01%, is addressed in the MSDS. It is used only as a bacteriostatic agent and is flushed from the resin during equilibration and preparation for use.

Recombinant Protein A.

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

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MATERIAL SAFETY DATA SHEET:
Recombinant Protein A, Revision 1 (Effective 12/23/2009)

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
Product Name:	Recombinant Protein A
Synonyms	rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A
Catalog No(s):	10-1001, 10-1501, 10-2001

SECTION 2 – COMPOSITION / INFORMATION ON INGREDIENTS

Purified Recombinant Protein A, derived from genetically modified *Escherichia coli.* Product is provided frozen in an aqueous buffer.

SECTION 3 – 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 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 (rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A)

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

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

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Recombinant Protein A (rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A)

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

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AGAROSE –(6% Highly Cross Linked)

There are no known toxic effects associates with this product, see attached supplier MSDS.

GE Healthcare

Material Safety Data Sheet

United States
English

Section 1. Chemical product and company identification

Product name Sepharose™ 6 Fast Flow, 1 L

Catalogue Number 17-0159-01

Material uses Industrial applications: Analytical chemistry. Research. Liquid chromatography.
Product type Liquid.
Validation date 16 October 2007
Print date 17 October 2007
Supplier GE Healthcare Bio-Sciences AB
SE-751 84 Uppsala
Sweden
+46 (0)18 612 0000

In case of emergency

US	ChemTrec (US)	1-800-424-9300
Canada	ChemTrec (US)	1-703-527-3887

2. Hazards identification

Physical state Liquid. [and Suspension.]
Odor Sweetish. Alcohol-like. [Slight]
OSHA/HCS status This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
Emergency overview WARNING !

FLAMMABLE LIQUID AND VAPOR. COMBUSTIBLE. CAUSES EYE IRRITATION. MAY CAUSE RESPIRATORY TRACT AND SKIN IRRITATION. CONTAINS MATERIAL THAT CAN CAUSE TARGET ORGAN DAMAGE.

Flammable liquid. Irritating to eyes. Moderately irritating to the skin and respiratory system. Keep away from heat, sparks and flame. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Avoid contact with eyes, skin and clothing. Contains material that can cause target organ damage. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

Routes of entry Dermal contact. Eye contact. Inhalation. Ingestion.

Potential acute health effects

Eyes Irritating to eyes.
Skin Moderately irritating to the skin.
Inhalation Moderately irritating to the respiratory system.
Ingestion No known significant effects or critical hazards.

Potential chronic health effects

Chronic effects Contains material that can cause target organ damage.
Carcinogenicity No known significant effects or critical hazards.
Mutagenicity No known significant effects or critical hazards.
Teratogenicity No known significant effects or critical hazards.
Developmental effects No known significant effects or critical hazards.
Fertility effects No known significant effects or critical hazards.
Target organs Contains material which causes damage to the following organs: blood, kidneys, the reproductive system, liver, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Inhalation Adverse symptoms may include the following:
respiratory tract irritation
coughing
Ingestion No specific data.
Skin Adverse symptoms may include the following:
irritation
redness

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Eyes	Adverse symptoms may include the following: pain or irritation watering redness
Medical conditions aggravated by over-exposure	Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (section 11)

3. Composition/information on ingredients

Name	CAS number	% by weight	Exposure limits
Ethanol	64-17-5	14 - 19	
Sepharose (highly cross-linked agarose)	9012-36-6	-	

Section 4. First aid measures

Eye contact	Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Get medical attention.
Skin contact	Wash with soap and water. Remove contaminated clothing and shoes. Get medical attention if irritation develops.
Inhalation	If inhaled, remove to fresh air. Get medical attention if symptoms appear.
Ingestion	Do not ingest. Get medical attention if symptoms appear.
Protection of first-aiders	No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Section 5. Fire fighting measures

Flammability of the product	Flammable liquid. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion. Runoff to sewer may create fire or explosion hazard.
Extinguishing media	
Suitable	Use dry chemical, CO_2, water spray (fog) or foam.
Not suitable	Do not use water jet.
Special exposure hazards	Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.
Hazardous combustion products	Decomposition products may include the following materials: carbon oxides
Special protective equipment for fire-fighters	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up	Stop leak if without risk. Move containers from spill area. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product. Note: see section 1 for emergency contact information and section 13 for waste disposal.
Small spill	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor.

Section 7. Handling and storage

Handling	Put on appropriate personal protective equipment (see section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Do not get in eyes or on skin or clothing. Do not breathe vapor or mist. Do not ingest. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use non-sparking tools. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by grounding and bonding containers and equipment before transferring

material. Empty containers retain product residue and can be hazardous. Do not reuse container.

Storage

Store between the following temperatures: 4 to 30°C (39.2 to 86°F). Store in accordance with local regulations. Store in a segregated and approved area. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Eliminate all ignition sources. Separate from oxidizing materials. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

Section 8. Exposure controls, personal protection

Product name	Exposure limits
Ethanol	**ACGIH TLV (United States).** IDLH: 3300 ppm **NIOSH REL (United States, 12/2001).** TWA: 1900 mg/m³ 10 hour(s). TWA: 1000 ppm 10 hour(s). **OSHA PEL (United States, 11/2006).** TWA: 1900 mg/m³ 8 hour(s). TWA: 1000 ppm 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 1900 mg/m³ 8 hour(s). TWA: 1000 ppm 8 hour(s). **ACGIH TLV (United States, 1/2006). Notes: 1996 Adoption Refers to Appendix A -- Carcinogens.** TWA: 1880 mg/m³ 8 hour(s). TWA: 1000 ppm 8 hour(s).

Recommended monitoring procedures

If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.

Engineering measures

Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Hygiene measures

Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal protection

Respiratory

Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Recommended: A respirator is not needed under normal and intended conditions of product use.

Hands

Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
1-4 hours (breakthrough time): butyl rubber, neoprene

Eyes

Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.
Recommended: safety glasses with side-shields

Skin

Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
Recommended: lab coat

Environmental exposure controls

Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Personal protective equipment (Pictograms)

Section 9. Physical and chemical properties

Physical state	Liquid. [and Suspension.]
Flash point	Closed cup: 38 to 43°C (100.4 to 109.4°F)
Color	solution : Colorless. / Suspension. : White.
Odor	Sweetish. Alcohol-like. [Slight]
Taste	Alcohol-like.
Volatility	14 to 19% (w/w)
Odor threshold	180 ppm
VOC	140 to 190 (g/l).
Ionicity (in water)	Non-ionic.

Solubility Easily soluble in the following materials: cold water and hot water.

Section 10. Stability and reactivity

Stability The product is stable. Under normal conditions of storage and use, hazardous polymerization will not occur.

Conditions to avoid Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.

Materials to avoid Reactive or incompatible with the following materials: oxidizing materials

Hazardous polymerization Will not occur.

Conditions of reactivity Highly flammable in the presence of the following materials or conditions: open flames, sparks and static discharge and heat.
Non-flammable in the presence of the following materials or conditions: shocks and mechanical impacts, oxidizing materials, reducing materials, combustible materials, organic materials, metals, acids, alkalis and moisture.
Not considered to be a product presenting a risk of explosion.

Section 11. Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Ethanol	LD50 Intra-arterial	Rat	11 mg/kg	-
	LD50 Intraperitoneal	Rat	3600 ug/kg	-
	LD50 Intravenous	Rat	1440 mg/kg	-
	LD50 Oral	Rat	7060 mg/kg	-
	TDLo Intracerebral	Rat	106 ug/kg	-
	TDLo Intravenous	Rat	0.5 g/kg	-
	TDLo Oral	Rat	6000 mg/kg	-
	TDLo Oral	Rat	5000 mg/kg	-
	TDLo Intraperitoneal	Rat	3000 mg/kg	-
	TDLo Intraperitoneal	Rat	500 mg/kg	-

Conclusion/Summary Not available.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
Ethanol	A4	-	-	-	-	-

Section 12. Ecological information

Environmental effects No known significant effects or critical hazards.

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Ethanol	Intoxication	Acute EC50 >100 mg/L	Daphnia	48 hours
	Intoxication	Acute EC50 9.3 mg/L	Daphnia	48 hours
	Physiology	Acute EC50 2 mg/L	Daphnia	48 hours
	Mortality	Acute LC50 13000 mg/L	Fish	96 hours
	Mortality	Acute LC50 >100 mg/L	Daphnia	96 hours
	Mortality	Acute LC50 >100 mg/L	Fish	96 hours

Conclusion/Summary Not available.

Biodegradability

Product/ingredient name	Test	Result	Dose	Inoculum
Ethanol	-	100 % - Readily - 20 days	-	-

Conclusion/Summary Not available.

Toxicity of the products of biodegradation The product itself and its products of degradation are not toxic.

Other adverse effects No known significant effects or critical hazards.

Section 13. Disposal considerations

Waste disposal

The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Waste stream

Code: D001
Classification: Ignitability

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

Section 14. Transport information

International transport regulations

Not classified.

Remarks

IATA Special Provision A 58 - Aqueous solutions containing 24% or less alcohol by volume is not subject to these regulations.

Section 15. Regulatory information

HCS Classification

Combustible liquid
Irritating material
Target organ effects

U.S. Federal regulations

United States inventory (TSCA 8b): All components are listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: Ethanol
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: Ethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard

Clean Water Act (CWA) 307: No products were found.

Clean Water Act (CWA) 311: No products were found.

Clean Air Act (CAA) 112 accidental release prevention: No products were found.

Clean Air Act (CAA) 112 regulated flammable substances: No products were found.

Clean Air Act (CAA) 112 regulated toxic substances: No products were found.

State regulations

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

Ingredient name	Cancer	Reproductive	No significant risk level	Maximum acceptable dosage level
Ethanol	No.	No.	No.	No.

United States inventory (TSCA 8b) **United States inventory (TSCA 8b):** All components are listed or exempted.

EU regulations

Risk phrases R10- Flammable.

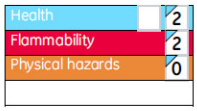
International regulations

International lists	**Australia inventory (AICS):** All components are listed or exempted. **China inventory (IECSC):** All components are listed or exempted. **Korea inventory (KECI):** All components are listed or exempted. **Philippines inventory (PICCS):** All components are listed or exempted. **Japan inventory (ENCS):** All components are listed or exempted.

Section 16. Other information

Label requirements FLAMMABLE LIQUID AND VAPOR. COMBUSTIBLE. CAUSES EYE IRRITATION. MAY CAUSE RESPIRATORY TRACT AND SKIN IRRITATION. CONTAINS MATERIAL THAT CAN CAUSE TARGET ORGAN DAMAGE.

Hazardous Material Information System (U.S.A.)



Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks Although HMIS® ratings are not required on MSDSs under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered mark of the National Paint & Coatings Association (NPCA). HMIS® materials may be purchased exclusively from J. J. Keller (800) 327-6868.

The customer is responsible for determining the PPE code for this material.

National Fire Protection Association (U.S.A.)

Indicates information that has changed from previously issued version.

History

Date of printing	17 October 2007	**Date of previous issue**	18 October 2006
Date of issue	16 October 2007	**Version**	5

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Agarose - Human Toxicity Excerpts [1] :

Result - ASIDE FROM AN OCCASIONAL ALLERGIC REACTION, THESE DRUGS CAN BE INGESTED IN LARGE AMT, WITH LITTLE DANGER OR DISTRESS EXCEPT FOR DIARRHEA, FLATULENCE, & RARELY FECAL IMPACTION. /GUMS, VEGETABLE/

Agarose - Minimum Fatal Dose Level [2]:

Result - PRACTICALLY NON-TOXIC: PROBABLE ORAL LETHAL DOSE (HUMAN) ABOVE 15 G/KG, MORE THAN 1 QUART FOR 70 KG PERSON (150 LB). /GUMS, VEGETABLE/

Agarose - Summary Toxicity Information [3]:

PAN Bad Actor Chemical (1.)	Acute Toxicity (2.)	Carcinogen	Cholinesterase Inhibitor	Ground Water Contaminant	Developmental or Reproductive Toxin	Endocrine Disruptor
NOT LISTED	NOT ACUTELY TOXIC	?	NO	?	?	?
KEY						
	Indicates high toxicity in the given toxicological category.					
?	Indicates no available weight-of-the-evidence summary assessment.					
NOTES.						
1	PAN Bad Actors are chemicals that are one or more of the following: highly acutely toxic, cholinesterase inhibitor, known/probable carcinogen, known groundwater pollutant or known reproductive or developmental toxicant. NOTE! Because there are no authorit					
2	The acute toxicity reported is of the pure chemical ingredient only and may not reflect the acute toxicity of individual pesticide products.					

4.2 Extractables and Leachables

Due to the legacy status of IPA 400HC and other than the Protein A leaching test described in section 3.3 above, Repligen has not tested IPA 400HC for extractables.

Note: IPA 400HC HAS BEEN MANUFACTURED AND MARKETED BY REPLIGEN FOR OVER 12 YEARS. IT HAS BEEN VALIDATED FOR USE IN THE MANUFACTURE OF REGULATED THERAPEUTICS APPROVED FOR HUMAN USE.

For IPA 400HC Repligen recommends that customers undertake characterization studies under their specific process conditions. As a guide, we can provide the following list of chemicals that are used during manufacture of IPA 400HC:

- Highly Cross Linked Agarose
- Sodium Metaperiodate
- Sodium Carbonate
- Sodium Borohydride
- Recombinant Protein A
- Thimerosal

5.0 Manufacturing Information:

5.1 Manufacturing Introduction

Repligen's IPA manufacturing operation is located at Repligen corporate headquarters, 41 Seyon Street, Waltham, Massachusetts, 02453, USA. The QA and QC operations are located at Repligen's Waverley Oaks facility, in Waltham Massachusetts. 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 audit.

IPA 400HC consists of Repligen's recombinant protein A™ (srPA50) which is covalently linked to 6% highly crossed linked agarose formulated to 52.5% ± 1% suspension in 0.01% Thimerosal preservative.

5.2 Manufacturing - 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

Repligen's Quality Policy reflects these needs and Repligen's firm commitment to meet or exceed customer expectations. This commitment to customer satisfactions is achieved through:

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

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

Repligen's quality system is currently certified by BSI America to ISO 9001:2008, see attached certification.

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 9001:2008

This is to certify that:

Repligen Corporation
41 Seyon St.
Waltham
Massachusetts
02453
USA

Holds Certificate No: **FM 535355**
and operates a Quality Management System which complies with the requirements of ISO 9001:2008 for the following scope:

The Scope of Repligen's Quality Management System is in support of the Protein A Products manufactured by Repligen and supplied to customers worldwide. The Quality Management System also supports the attendant services necessary for fulfillment of customer orders.

For and on behalf of BSI:

President, BSI America, Inc.

Originally Registered: **12/18/2008** Latest Issue: **11/11/2009** Expiry Date: **12/17/2011**

Page: 1 of 1

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.
Americas Headquarters: 12110 Sunset Hills Road, Suite 200, Reston, VA 20190, USA.

5.3 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 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.

Repligen's 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.

5.4 Manufacturing Facilities

Repligen's BioProcessing Manufacturing facility consists of 3 main areas.

5.4.1 Fermentation

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

5.4.2 Recovery

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

5.4.3 Clean Room

The clean room 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.

5.4.4 Contract fermentation:

Repligen uses contractors for certain fermentation operations, including the fermentation of srPA50. Contractors are held to the same quality standard as Repligen's own manufacturing facilities, and are audited annually. Maintaining a secondary fermentation site is part of Repligen's formal Business Continuity Management (BCMS) strategy.

5.4.5 Shipping:

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

5.5 Manufacturing Control: (See also Quality Assurance standards section).

- **Training:** Manufacturing is performed by qualified and trained operators. Training documentation is maintained by Quality Assurance.
- **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 verified and released by QA prior to use in manufacturing.
- **Process Change Control:** Manufacturing process changes are governed by the Repligen change management procedures. See Quality Assurance section
- **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 ASTM Type I Reagent Grade Water, but with the addition of low endotoxin and bioburden specifications, table 6. Water quality is routinely monitored by Repligen Quality Control.

Table 6. Repligen Water Specifications as Compared With ASTM, USP Purified and WFI.

	ASTM Type I	USP Purified water	WFI	Repligen Specification
Conductivity	0.05 µS/cm	1.3µS/cm	1.3µS/cm	0.01 mS/cm
LAL	N/A	N/A	< 0.25 EU/mL	0.5 EU/mL
Bioburden	N/A	100 cfu/mL	0.1 cfu/mL	10 cfu/mL
pH	N/A	N/A	5 – 7	5 – 7
TOC	0.1 ppm	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 7.

Table 7. 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

5.6 Manufacturing - IPA 400HC

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 ≥98% 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 still 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 the storage buffer and preservative. The srPA50 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 with 0.01% Thimerosal added as a bacteriostatic agent (18.5%±1% ethanol may be substituted by custom request). The slurry is adjusted to contain 52.5% 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.

5.7 Manufacturing - 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.

The IPA 400HC release tests are as follows:

- Reconciliation and Inspection: Physical count to verify quantities, and inspection of container/label integrity.
- Appearance: Visual inspection to ensure conformity of appearance (White suspension, ~50% fill)
- Determination of Immobilized rProtein A 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 IPA 400HC is measured by ELISA. The ELISA has a sensitivity of 2ng rProtein A per mL of supernatant, which corresponds to approximately 2ppm in an eluted antibody. The specification is ≤ 5 ng/mL.
- Determination of rProtein A hIgG binding: The static binding capacity is determined using human polyclonal IgG. The specification is 40 ± 3 mg of hIgG per mL of resin.

5.8 Manufacturing: IPA 400HC Sample Certificate of Analysis

RepliGen

Repligen Corporation
41 Seyon Street
Building #1, Suite 100
Waltham, MA 02453
Telephone: 781-250-0111
Telefax: 781-250-0115

Certificate of Analysis

PRODUCT: Immobilized rProtein A™ (IPA-400HC)
PRODUCT CODE: 10-2500
PRODUCT LOT: RNXXXXX

DATE MANUFACTURED: Month dd, YYYY
STORAGE CONDITIONS: 4 - 8°C

Immobilized rProtein A™ (IPA-400HC) consists of RepliGen's recombinant protein A covalently coupled to 6% Sepharose® FF. A unique immobilization chemistry provides high hIgG binding capacity, low ligand leakage and stability under a wide range of pH and buffer conditions.

Form: 50% slurry containing 0.01% Thimerosal as a preservative

TEST / METHOD	SPECIFICATION	RESULT
Human IgG Binding Capacity The capacity for binding human polyclonal IgG is determined in an assay performed in a batch mode, with the IgG loaded at pH 7.4, and eluted with 0.2M glycine pH 2.0. The eluate is assayed for protein concentration using a spectrophotometric assay at 280nm.	40 ± 3mg hIgG/mL	hIgG/mL
rProtein A™ Leakage The amount of leaked rProtein A™ that would contaminate an antibody preparation purified using Repligen Immobilized rProtein A™ is measured by an ELISA. The ELISA has a sensitivity of 0.5ng rProtein A™ per mL of supernatant, which corresponds to a level of contamination of approximately 0.01 ppm in an eluted antibody at the maximum binding capacity.	<5ng rProtein A™/mL	ng rPA™/mL

Quality Affairs

QA-FM-1358-01

6.0 User Instructions:

These user instructions provide general guidance on the basic practices associated with the use of agarose bead based Protein A affinity resins for the purification of monoclonal antibodies. As IPA 400HC is based on GE's Sepharose 6FF bead and Repligen's recombinant protein the well established user practices apply. These instructions are based on those well established practices as detailed in instructions for other Protein A Sepharose affinity resins. For the full original text refer to the references[4].

Included sections are:

- Column Packing
    - Slurry Preparation
    - Packing Evaluation
- Method design, optimization and screening
- Scale Up
- Optimization
- Processing and Maintenance
    - Regeneration
    - Cleaning-in-place (CIP)
    - Sanitization
    - Storage

Column Packing

Slurry preparation for and packing of smaller scale developmental columns is typically a simple manual process; although any bed height can be created

the accepted default standard is 20cms. A generic protocol for manually packing a small column is as follows:

Materials

- Appropriate volume of IPA 400HC depending on column diameter and bed height. For small columns this typically in the 10's of milliliter range and can be accurately calculated using the following formula:

  $v=(r^2 x \pi)b$ *where:*

  v= volume of IPA in milliliters

  r= radius of the column in centimeters

  b= bed height in centimeters

- Glass filter funnel
- Scoop
- Erlenmeyer flask (of appropriate volume)
- Beaker (of appropriate volume)
- 20% EtOH in water
- 250 ml 20% EtOH + 0.25 M NaCl

Step 1 – Resin Washing

Equilibrate the resin slurry required to room temperature. Pour the IPA 400HC resin into the funnel and wash into the flask as follows:

- First with a 2x volume of 20% EtOH
- Followed by a 4x volume of 20% EtOH + 0.25 M NaCl

Step 2 - Slurry Preparation

The washed resin is now suspended in 20% EtOH + 0.25 M NaCl. Pour the washed IPA 400HC resin into a beaker and add an equivalent volume of 20% EtOH + 0.25 M NaCl (i.e. for 50mls resin add 50mls 20% EtOH + 0.25 M NaCl)

Step 3 - Column Packing

Materials

- Column (various)
- Pump
- Packing reservoir
- Syringe, tubing and glass rod

Assemble the column - following the column manufacturers instructions, ensure that all parts are clean and intact.

1. Connect the packing reservoir to the column.
2. Inject 0.25 M NaCl, 20% ethanol into the bottom of the column with a syringe; ensure that there are no trapped air bubbles. Close the tubing with a stopper
3. Flush the column and reservoir with 0.25 M NaCl, 20% EtOH, leaving a few ml at the bottom and mount the column vertically on a laboratory stand.

Pack IPA 400HC – As the IPA 400HC resin is based on the GE Sepharose 6FF base bead these agarose bead packing instructions are well established[5].

IPA 400HC is supplied pre-swollen in 0.01% Thimerosal preservative solution. Prepare the IPA 400HC by removing the storage solution and replace it with starting buffer in a ratio of 75% settled IPA 400HC to 25% buffer. The starting buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow after packing has been completed.

Pour the slurry into the column, for best results the column should be filled in one time at a smooth steady rate. Typically this is accomplished by pouring down a glass rod held against the wall of the column that helps prevent the introduction of air bubbles.

1. Fill the remaining column space and reservoir with 0.25 M NaCl in 20% EtOH. Place the lid on the packing reservoir and connect it to the pump.
2. Open the column outlet and start the packing by pumping 0.25 M NaCl, 20% EtOH through the column at a flow rate of 1.5 X the maximum process flow rate until the bed height stabilizes, this should take no more than 5 minutes.
3. Switch off and disconnect the pump. Close the column outlet.
4. Remove the packing reservoir (this is best done over a sink or drain). Refill the column to the top with 0.25 M NaCl, 20% EtOH.
5. Wet the column adaptor by submerging the plunger end in 0.25 M NaCl, 20% ethanol, and drawing through with a syringe. Ensure that all bubbles have been removed.

6. Insert the adaptor into the top of the column, taking care not to introduce air bubbles.
7. With the adaptor outlet open, push the adaptor into the column and down onto the resin bed, allowing the 0.25 M NaCl, 20% EtOH to displace any air remaining in the tubing.
8. Lock the adaptor in place, connect it to the pump, open the column outlet and continue packing at a flow rate equivalent to the process flow rate (≤ 3 bar) for 3 minutes.
9. Mark the position of the top of the bed height on the column cylinder wall. Stop the pump, close the column outlet and reposition the adaptor to approximately 1 mm below the marked bed height position.

Use of an adaptor

Adaptors should be fitted as follows:

1. After the gel has been packed as described above, close the column outlet and remove the top piece from the column.
2. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
3. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
4. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump and column and the sample application system (LV-3 or LV-4).
5. Slide the plunger slowly down the column so that the air above the net and in the capillary tubing is displaced by eluent. Valves on the

inlet side of the column should be regularly turned to ensure that all air is removed.

6. Lock the adaptor in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adaptor on the gel surface as necessary.

Packing large-scale columns - General recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Please refer to the relevant column instruction manual carefully.

IPA 400HC is easy to pack since its rigidity allows the use of moderately high flow rates IPA 400HC is typically packed at up to a maximum velocity of 300cmhr^{-1} or pressure < 3 bar.

Note: As the columns increase in diameter the packing flow rate decreases, at packing flow rates below 150cmhr^{-1} there is generally little impact, at higher flow rates a 3 fold increase in column diameter can increased packing pressure approximately 2 fold.

In general there are three suitable types of packing methods:

- Pressure packing (for columns with moveable adaptors).
- Combined pressure/suction packing (for medium sized columns with fixed bed heights).
- Suction packing (for large columns with fixed bed heights).
- Hydraulic pressure packing.

How well the column is packed will have a major effect on the performance of the resin and the purity and yield of the purification process. Guidelines are

given for determining the optimal packing flow rates for different column designs columns with specific design features like adaptors and fixed bed heights.

Determining the optimal packing pressure

The optimal packing pressure/flow rate is dependent on column size, type, desired bed height, packing solution and temperature. The optimal packing flow rate must therefore be determined empirically for each individual system. Generically this is done as follows:

1. Calculate the exact amount of IPA 400HC needed for the slurry (this is especially important for columns with fixed bed heights). Extra resin is required to allow for settling of the bed allow approximately 1.15L of resin slurry per 1 liter of packed bed.
2. Prepare the column per the column instructions.
3. Begin packing the column at a low flow rate (e.g. 30% of the expected max process flow rate), record the flow rate and back pressure when the bed is completely packed and the pressure has stabilized.
4. Increase flow rate recording both flow rate and pressure drop in a stepwise manner always allowing the pressure to stabilize at each step.
5. Continue recording flow and pressure until the maximum process flow rate has been reached. This reached when the pressure flow curve levels off or the maximum column pressure is reached.
6. Plot pressure against flow rate.

The optimal packing pressure is about 70% of the maximum pressure. From the packing pressure point on the curve, draw a straight line to zero. The maximum operational pressure should be <70% of the packing pressure. From the straight line, the maximum operational flow rate can be found.

Pressure Packing – typically for columns supplied with a movable top flow plate (e.g., GE BPG™; Millipore Vantage™ and Quickscale™) are packed by conventional pressure packing where packing solution is pumped through the settling chromatographic bed at a constant back pressure. Specific packing instructions and pressure flow curves are generally provided by the column manufacturers and can be matched with each resins pressure flow properties to develop a robust packing protocol for each column/resin combination. Generically the steps are as follows:

1. Make sure no air is trapped under the bottom bed support by pumping packing buffer through it from below. Excess liquid in the column can be removed by connecting tubing to the suction side of a pump. Leave about 2 cm of liquid in the column.
2. Mix the packing buffer with the medium to form a 50% slurry (settled bed volume/slurry volume = 0.5). Pour the slurry into the column. Insert top distributor plate the adaptor and lower to the surface of the slurry, making sure no air is trapped under the plate and secure in place.
3. Fill the adaptor inlet with packing solution.
4. Connect a pump and a pressure meter; apply a flow that gives the proscribed back pressure (typically about 0.1 bar). When the bed has settled, run for a few minutes, close the valve and stop the pump. Lower the plate down to the top of the bed.

5. Start the pump and apply a flow that gives the desired packing pressure. Keep the pressure constant during packing and check the pressure at the column inlet. **Never** exceed the pressure limit for column or medium. Run for at least 15 min.
6. When the bed has stabilized, mark the bed height, close the valve and stop the pump.
7. Disconnect the column inlet tubing and replace it with tubing leading to waste, push the top plate adaptor down to approximately 3 mm below the mark on the column tube. The packing solution will flush the adaptor inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Combined pressure/suction packing – typically these columns have a fixed bed height of 15 cm. It is packed by a combined pressure/suction technique. Follow the column manufacturer's instructions, which generically include:

1. Fitting an extra column section on top of the column tube as a packing reservoir.
2. Pour water or packing buffer into the column making sure that there is no air trapped under the bottom bed support. Leave about 2 cm of liquid in the column.
3. Pour the slurry into the column. Stir gently to give an homogeneous slurry. Add buffer until level with the upper rim and secure the lid in place.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the

column inlet. Never exceed the pressure limit for the column or medium.

5. When the bed has stabilized, the top of the bed should be exactly level with the top of the column tube. Switching the valve takes the buffer tank off line the inlet pump is now connected to the outflow side of the column. The packing buffer is re-circulated in the system. If, when stabilized, the packed bed is not exactly level with the top of the column, add or remove slurry.
6. Keep the pump running, disconnect the column inlet and direct it to waste. The packing solution in the packing section is removed by suction through the bed.
7. Remove the packing reservoir section.
8. When the packing solution is within 5–8 mm of the bed surface stop the pump. This final operation should be completed as quickly to prevent bed expansion.
9. Start pumping buffer with upward flow through the column to remove any air bubbles.

Suction Packing – typically for columns with fixed end pieces. These columns are packed by suction, i.e. by sucking packing solution through the chromatographic bed at a constant flow rate.

1. Fit a packing device on top of the column tube.
2. Pour water or packing buffer into the column making sure that there is no air trapped under the bottom bed support. Leave about 2 cm of liquid in the column.
3. Mix the packing buffer with the medium to form a 50% slurry (settled bed volume/slurry = 0.5). Pour the slurry into the column.

4. Connect the column outlet valve to the suction side of a pump and start packing the bed by suction through the bed at the predetermined flow rate. Keep the flow rate constant during packing.
5. When the bed has stabilized, the top of the bed should be just below the junction between the column and the packing device.
6. Just before the last of the packing solution enters the packed bed, stop the pump and quickly remove the packing device and replace it with the lid. This final operation should be completed quickly to prevent bed expansion when the flow stops.
7. Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

Hydraulic Packing - is for columns supplied with a hydraulic function GE INdEX™ and FineLine™; Novasep Prochrom® DAC. In these systems an automated hydraulic systems controls packing as the adaptor is lowered into position at the correct pressure. The adaptor is pushed down by a constant hydraulic pressure, forcing packing buffer through the slurry and compressing it so that a packed bed is gradually built up.

The quantity of medium required when packing IPA 400HC by hydraulic pressure is approximately 1.15L of resin slurry per 1 liter of packed bed. Generically packing is completed as follows:

1. Make sure that there is no air trapped under the bottom bed support, by pumping packing buffer through it from below. Leave about 2 cm of liquid in the column.
2. Pour the slurry into the column. Fill the column with packing solution up to the top of the tube allowing the medium bed to settle just below the top of the column tube.

3. Put the adaptor in a resting position in the column tube and lower the lid and secure it in place.
4. Connect a pump to the inlet, to start the packing, applying a predefined constant hydraulic packing pressure. When packing IPA 400HC in this type of column pack the bed to less than the recommended operational pressure.
8. When the adaptor has reached the surface of the settled bed, continue to run the pump until the adaptor has been lowered fractionally into the packed bed (depending on the column manufacturer's instructions)

Packing Efficiency Assessment

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, prior to re-use and if there is an observed deterioration in separation performance. The efficiency of a packed column is expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). These values are easily determined by applying a sample such as 1% acetone solution to the column and using water as eluent. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent. It is important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results. A sample volume of less than 2.5% of the column volume and the flow velocity between 15 and 30 cm/h will give the most optimal results.

Method for measuring HETP and Asymmetry

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

Conditions

- Sample volume: 1.0% of the bed volume
- Sample conc.: 1.0% v/v acetone
- Flow velocity: 20 cm/h
- UV: 280 nm, 1 cm, 0.1 AU

Calculate HETP and A_s from the UV curve (or conductivity curve if NaCl is used as sample) as follows:

$$HETP = L/N$$

$$N = 5.54(Ve/Wh)^2$$

where L = Bed height (cm)

N = Number of theoretical plates

Ve = Peak elution distance

Wh = Peak width at half peak height

Ve and Wh are in the same units.

To facilitate comparison of column performance the concept of reduced plate height is often used.

Reduced plate height is calculated:

HETP/d where

d is the mean diameter of the bead. As a guideline, a value of <3 is normally acceptable.

For a well-packed efficient column the peak should be symmetrical, and the asymmetry factor as close as possible to 1 (values between 0.8–1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation:

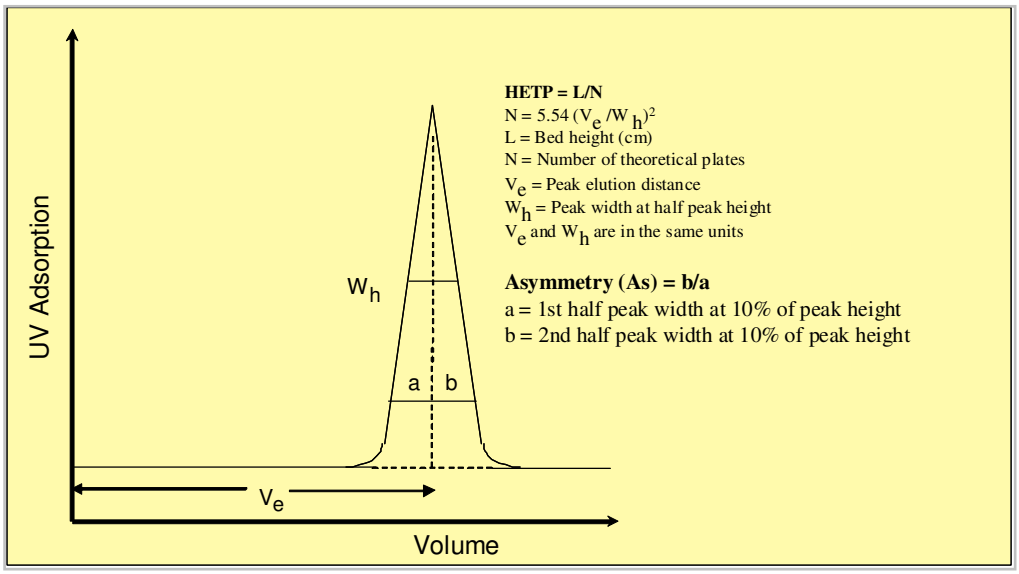
A_s = b/a where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height.

Figure 2 shows a UV trace for acetone in a typical test chromatogram in which the HETP and As values are calculated.

Figure 3. HETP and A_s Calculations.



Performance testing of packed IPA 400HC columns.

To check the quality of the column packing users should test according to the specification of the manufacturer [6] of the beads, in the case of IPA 400HC this is the GE Sepharose 6FF agarose. An efficiency test should be performed to determine the theoretical plate number and peak asymmetry factor.

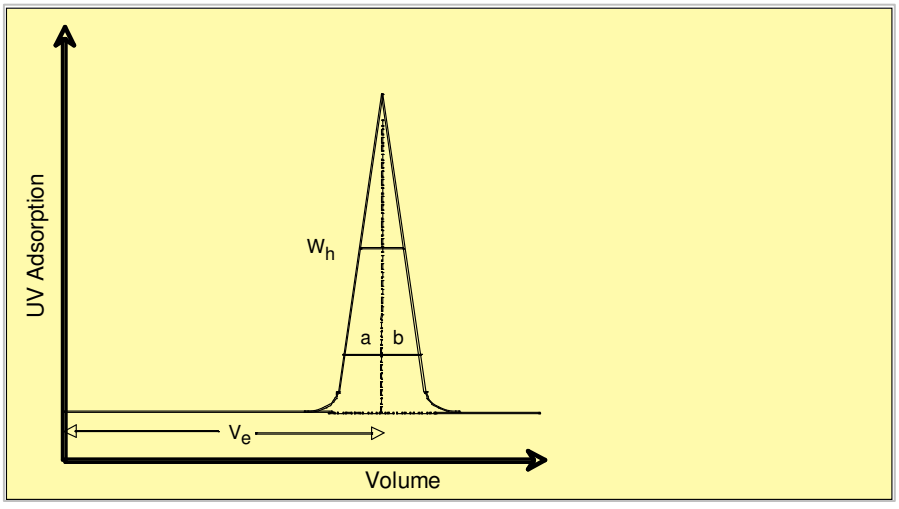
Using distilled water as an eluent and 1% (v/v) acetone in distilled water as the sample. The column is tested by pumping 200 µl of acetone (20mg/ml) through the column at a flow typically between 20 and 100cmhr^{-1}. Calculations for both plate number using the formula: $N/m = 5.54\ (VR/Wh)^2 \times 1000/L$ and peak asymmetry factor (A_s) by the formula: $A_s = b/a$ are shown in Figure 3.

If the column is packed according to the instructions described above typical values obtained for IPA 400HC should be:

Number of theoretical plates > 3,000

Peak asymmetry 0.8-1.5

Figure 4. Calculation of Packing Performance



Method Design and Optimization

As with most affinity chromatography media, IPA 400HC offers high selectivity for Monoclonal antibodies, which reduces the impact of process related parameters such as sample load, flow rate, bead size and bed height on resolution. The primary aim of method optimization is to establish the conditions that bind the highest amount of target molecule, in the shortest time and with the highest product recovery.

Specificity and affinity

The degree to which Protein A binds to IgG varies with respect to both the origin and antibody subclass [7].

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.

Method screening

Because the affinity of Protein A affinity resins like IPA 400HC varies for antibodies of different species, classes and subclasses varies, initial

screening should be conducted under a broader range of conditions that will bind the largest diversity of antibodies and highlight potential interference between the target antibody and possible contaminating antibodies. A good general approach to evaluating primary mAb binding to IPA 400HC is to start with high pH and high salt conditions, then elute them in a reducing linear salt/pH gradient. It is important to make certain that the antibody is stable under the elution conditions in order not to lose biological activity.

General Screening Recommendations

Example of suitable buffers:

- Buffer A: 0.05 M boric acid, 1.0 to 2.0 M NaCl, pH 9.0
- Buffer B: 0.05 M sodium citrate, 0.3 M NaCl, pH 3.0

Experimental conditions:

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

Conditions can be subsequently modified to provide the best purification performance. High salt concentration and high pH will often increase dynamic binding capacity, even for antibodies, decreasing salt concentration and/or pH during binding may change contaminant mAb binding. This may also increase the dynamic binding capacity since more binding sites will be available for the target antibody. It may also increase selectivity in the system. The balance between selectivity and capacity must be defined with respect to the nature of the feed, i.e. presence of contaminating antibodies and the purity requirement in the eluted product. When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column. Low pH values tend to encourage denaturation and aggregation of antibodies.

Scale Up

After optimizing the antibody fractionation at laboratory scale, the process can be scaled up. For this, some parameters will change while others remain constant.

- Bed volume changes according to required binding capacity and process load.
- Column diameter changes as a function of bed volume to obtain a bed height of approximately 20 cm so that high flow rates and high dynamic capacity can be used.
- Linear flow rate remains constant during sample application to ensure that residence time is not shorter than that established in the small-scale experiments. The residence time is equal to the bed

height (cm) divided by the mobile phase velocity (cm/h) applied during sample loading.

- Keep sample concentration and gradient slope constant.
- The larger equipment needed when scaling up may cause some deviations from the optimized method at small scale. Different lengths and diameters of outlet pipes can cause zone spreading check the buffer delivery system and monitoring system for time delays or volume changes.

Optimization of Throughput

The optimal flow rate is that which gives the highest throughput in terms of amount of antibody processed per time unit and volume of medium.

This is achieved by defining the highest sample load over the shortest sample application time with the least amount of product loss. Frontal curve analysis provides this information. Since the dynamic binding capacity is a function of the linear flow rate applied during sample application, the breakthrough capacity must be defined over a range of different flow rates. The optimal flow rate is that which gives the highest throughput in terms of amount of antibody processed per time unit and volume of medium.

Removal of leached Protein A from final product

Leakage of Protein A from IPA 400HC is generally very low. However, in many monoclonal applications it is a requirement that leached Protein A is eliminated from the final product. In a multi-step purification process this is usually achieved through the use of a 2nd and/or 3rd chromatographic step.

Size exclusion chromatography can be applied for removal of Protein A-IgG aggregates by conducting the separation under moderate pH conditions. The large IgG-Protein A complexes that are formed will elute early from the column.

Cation exchange chromatography is an effective tool for removing residual Protein A, especially when the particular monoclonal has strong cation exchange binding characteristics. The run is conducted at a pH in which the antibody is known to dissociate from Protein A. Protein A binds poorly to cation exchangers and will elute early in the gradient.

Anion exchange chromatography can also be used to reduce leached Protein A contamination. It is best suited to antibodies that are weakly retained on anion exchangers. Because of the strong anion exchange binding characteristics of Protein A, Protein A-IgG complexes tend to be more strongly retained than noncomplex antibodies.

Processing and Maintenance

Cleaning-in-place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the resin and hardware. The accumulation of these contaminants may affect subsequent performance of the purification system or allow unwanted, potentially immunogenic, contaminants into the bulk API. If the fouling is severe, it may block the column, increase back pressure and reduce flow rate. Regular CIP prevents the build up of these contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of IPA 400HC.

CIP protocols

The following CIP protocols are intended as a starting point cleaning protocols specific for a given feed material. Typically, CIP is conducted every 5 cycles but this will ultimately depend on the nature of the feed material. Different contaminants require different or even combine CIP protocols. Severe fouling will require specific protocol development.

Precipitated or denatured substances:

- Wash with 2 column volumes of 6 M guanidine hydrochloride[1] 10 mM NaOH[2], 0.1 M H_3 PO_4 or 50 mM NaOH in 1.0 M NaCl or 50 mM NaOH in 1.0 M Na_2SO_4[(3)].
- Wash immediately with at least 5 column volumes of 0.2µm filtered binding buffer at pH 7–8.
- Reverse flow direction.

Hydrophobically bound substances

- Wash the column with 2 column volumes of a non ionic detergent[1] (e.g. conc. 0.1%).
- Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7–8.
- Reverse flow direction.

OR

- Wash the columns with 3–4 columns volumes of 70% ethanol[1] or 30% isopropanol[1].
- Wash immediately with at least 5 columns volumes of sterile filtered binding buffer at pH 7–8.
- Reverse flow direction.

- Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

Notes:

1. Apply for an approximate contact time of 10 minutes on the column.

2. Apply for an approximate contact time of 30 minutes on the column.

3. Apply for an approximate time of 16 minutes on the column.

Sanitization

Sanitization protocols are used to reduce microbial contamination of the resin bed, often prior to storage. Effective sanitization prevents the build up of microorganisms that can lead to endotoxins contamination or a fouled resin bed. There are 3 common approaches to sanitization protocols:

Equilibrate the column with a solution consisting of 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours, and then wash with at least 5 column volumes of sterile binding buffer.

OR

Equilibrate the column with a solution consisting of 0.1 M acetic acid and 20% ethanol. Allow to stand for 1 hour, and then wash with at least 5 column volumes of sterile binding buffer.

OR

Equilibrate the column with 70% ethanol[1]. Allow to stand for 12 hours, then wash with at least 5 column volumes of sterile binding buffer.

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

Storage

Unused media can be stored in the container at a temperature of +2 to +8 °C. Ensure that the screw top is fully tightened. Packed columns should be equilibrated in binding buffer containing 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

Further information

Please read these instructions carefully before using IPA 400HC media. For further information using this product please visit www.repligen.com, OR contact our customer service specialists for more information or to schedule and appointment with Repligen's Applied Customer Engineering (ACE) Team for on site technical support and problem solving.

7.0 Bibliography

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2) www.toxnet.com; [Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason. Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, 1976., p. II-6] **PEER REVIEWED**

3) www.pesticideinfo.org; Extracted From the Pesticide Action network (PAN) Pesticides Database 2009

4) rmp Protein A Sepharose Fast Flow 71-5017-20; GE (Amersham Biosciences)

5) Ion Exchange Media (Instructions 56-1191-00 AG) ; GE (Amersham Biosciences)

6) Sepharose 6 Fast Flow (Data File 18-1020-52 AC) GE (Amersham Biosciences)

7) Purification Tools for Monoclonal Antibodies, Ch 9; Pete Gagnon

Important Information

IPA 400HC is a trademark of Repligen Corporation.

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