AVIPure® HiPer™ Phospholipid Affinity Resin

User Guide

TD0145 OCT2026

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Abbreviations

C	celsius
CCCF	clarified cell culture fluid
cm	centimeter
CV	column volumes
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
LVV	lentivirus vector
M	molar
$MgCl_2$	magnesium chloride
mL	milliliter
mM	millimolar
NaCl	sodium chloride
NaOH	sodium hydroxide
PL	phospholipid
psi	pounds per square inch
TFDF®	Tangential Flow Depth Filtration®
TFF	tangential flow filtration
µm	micrometer or micron

1. Introduction

AVIPure® HiPer™ Phospholipid (PL) Affinity Resin is an alkali-tolerant resin originally developed for purification of enveloped viral particles, such as lentiviral vectors (LVV), directly from clarified cell culture fluids (CCCF). This resin can also be used for capture of other enveloped particles such as exosomes and extracellular vesicles.

2. About This Document

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

Table 1. Explanation of User Attention Phrases

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

Table 2. Safety Precautions

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eyewear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	This product is shipped in an 18 % – 20% ethanol solution, with 0.1% acetic acid. It is flushed from the resin during equilibration and preparation for use. Follow all local regulations for safe disposal.
WARNING 	Flammable liquid and vapor. • Keep away from heat, sparks, open flames, and hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/ international regulations.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

3. Key Performance Attributes

Key performance attributes of AVIPure HiPer PL Affinity Resin include:

- Captures particles with a phospholipid envelope; free and truncated VSV-G flows through
- Low (300 mM) NaCl elution at neutral pH
- High purity (HCP ≥2 log reduction)
- High infectivity yield (~2× IEX membranes)
- Fast operating flow rates (residence times of 30 – 60 seconds or less)
- Clean with 0.5 M NaOH

Table 3. Performance Characteristics of AVIPure HiPer Phospholipid Affinity Resin

Category	Description
Base matrix	Cross-linked polymethacrylic polymer
Particle size (d50v)	50 µm
Ligand	Alkali-tolerant peptide (animal-free)
Coupling chemistry	Epoxide
Binding capacity	50 CV challenge using clarified cell culture fluids; potentially higher for high titer, clean harvests
Buffer compatibility	Stable to all commonly used aqueous buffers, including 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents
Solvent compatibility	Water, alcohol (0% – 20% v/v), acetonitrile, 1 – 2 M acetic acid, other common organic solvents
pH stability	1 – 13
Cleaning-in-place stability	0.1 – 0.5 M NaOH
Pressure/flow[a]	>600 cm/h at 2 bar
Maximum packing pressure (ΔP)[a]	2.8 – 3 bar
Temperature stability	2°C – 40°C
Delivery conditions	18% - 20% ethanol with 0.1% (20 mM) acetic acid[b]
Storage	2°C – 8°C, 18% – 20% ethanol with 0.1% (20 mM) acetic acid[b]; do not freeze

[a]10 × 10 cm OPUS® column
[b]This mitigates potential hydrolysis of the ligand, leading to longer shelf life.

4. Process Development

Optimal conditions for purification of LVV using AVIPure HiPer PL Affinity Resin must be determined empirically for each source. Some general process development recommendations for identification of optimal process conditions are summarized in Table 4. For the most up-to-date application notes, please refer to repligen.com.

The resin should be operated at a flow velocity that maintains a pressure drop across the column below the maximum packing pressure (2.8 – 3 bar). Factors that influence maximum flow velocity include viscosity of the running buffer and turbidity of the load solution. The maximum flow velocity will vary depending on the feed stream. When loading feed material for columns with an internal diameter (ID) of up to 12 cm and a 10 cm bed height (BH), the evaluation should be started at 600 cm/h. Users can increase flow velocity, provided the pressure drop over the column stays below the maximum packing pressure.

Internal process development was done using HEPES buffer, but different buffer systems such as Tris or Bis-Tris propane can be used provided the conductivity remains comparable to the recommended levels during loading and elution.

Table 4. Recommended Starting Purification Protocol for AVIPure HiPer PL Affinity Resin in Columns ≤12 cm ID x 5 cm BH

Step	Column Volumes	Residence Time, minutes	Suggested Buffer
Sanitization[a]	15	1	0.1 – 0.5 M NaOH
Equilibration	8	0.5	50 mM HEPES, 50 mM NaCl, pH 7.5
Load	50	0.5	Section 4.1
Wash	10	0.5	Equilibration buffer
Elution	5	0.5	50 mM HEPES, 300 mM NaCl, pH 7.5 (Section 4.2)
Strip	5	0.5	50 mM HEPES, 1 M NaCl, pH 7.5
CIP[a]	15	1	0.1 – 0.5 M NaOH
Re-equilibration	8	0.5	Equilibration buffer
Long-term storage	3	1	18% – 20% ethanol with 0.1% (20 mM) acetic acid at 2°C – 8°C

[a]Total contact time should be 15 minutes. A static hold can also be used.

4.1 Equilibration and Binding

While formal turbidity studies have not been completed, a load turbidity of 5 NTU or less should be targeted. All load material should be filtered through a 1 µm filter prior to loading.

Binding of LVV and exosomes to AVIPure HiPer PL Affinity Resin has been demonstrated with buffers at near-neutral pH (7 – 7.5) with a conductivity similar to the CCCF (≤12 mS/cm). Capacity is partially dependent on the quality and titer of the CCCF. A 50 CV load will generally provide good recovery with little breakthrough, though a clean feed with a high titer may afford 100 CV or higher loading without loss in the flow through.

The capacity of the resin is approximately 5×10^8 transduction units (TU)/mL resin, or 1×10^{10} or higher *total* phospholipid particles/mL. If the concentration of TU and total particles in solution is not well understood, the capacity can be determined by monitoring another marker, such as p24, at points during the flow through, starting with an early fraction to determine a baseline of free marker and continuing throughout the load.

Fractionate and collect the flow through during initial runs to understand capacity and breakthrough.

4.2 Elution

Targets can be eluted from the AVIPure HiPer PL Affinity Resin with the following recommended buffers:

- 50 mM HEPES, 300 mM NaCl, pH 7.5
- 50 mM HEPES, 112 mM arginine-HCl, 150 mM NaCl, pH 7.5
- 50 mM HEPES, 75 mM $MgCl_2$, 150 mM NaCl, pH 7.5

If yield is low and loss is not observed in the flow through, particles may be remaining on the column after elution. Typically, these are aggregated or otherwise non-infective material. Assessing the particle size in the harvest, elution, strip, and neutralized CIP can provide insight into optimizing product recovery.

4.3 Cleaning-in-Place and Sanitization

AVIPure HiPer PL Affinity Resin is an alkali-tolerant resin, enabling the use of NaOH up to concentrations of 0.5 M. A cleaning-in-place (CIP) regime of 0.1 – 0.5 M NaOH exposure for 15 minutes per cycle ensures consistent chromatographic performance. Neutralize columns prior to storage.

4.4 Storage

AVIPure HiPer PL Affinity Resin is shipped stored in 18% – 20% ethanol with 0.1% (20 mM) acetic acid, which helps prevent hydrolysis of the ligand and loss of capacity over time. Keep unused columns at 2°C – 8°C. Do not freeze. Following sanitization and neutralization, store columns at 2°C – 8°C in 18% – 20% ethanol with 0.1% acetic acid.

5. Troubleshooting

Field application scientists are available to assist in your process development. Contact your account manager or regional customer service.

Table 5. Troubleshooting

Observation	Issue	Correction
Early breakthrough	Load conductivity too high	Dilute load to a conductivity at or below 12 mS/cm.
	High amounts of debris in load	Cleaner feeds such as those that have undergone tangential flow filtration (TFF) or produced in an intensified Tangential Flow Depth Filtration® (TFDF®) process will enable higher capacity for infectious particles.
Low yield	Overloading	Flow-through should be fractionated and collected for analysis to understand effective capacity. Start with a 50 CV load for initial testing and increase from that point to avoid loss in flow through.
	Aggregation	Particles can become aggregated and remain on the column after elution. Collecting and analyzing the strip can provide helpful insight for process optimization.
High column pressure	Unfiltered sample	Filter the sample prior to running on the column. Aim for turbidity of 5 NTU or lower.
	Incompatible flow rate	Begin with 600 cm/h flow velocity and increase as appropriate. Factors that influence maximum flow velocity include viscosity of the running buffer and turbidity of the load solution. The maximum flow velocity will vary depending on the feed stream.

6. Ordering Information

AVIPure HiPer PL Affinity Resin can be ordered in OPUS pre-packed columns with a maximum bed height of 10 cm. Contact your account manager or your regional customer service using the email addresses below to place an order:

US: customerserviceUS@repligen.com
EU: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Table 6. Product List: Screening Resin and Off-the-Shelf OPUS PD-Scale Columns

Volume	Description	Part Number
5 mL	Bulk resin for high-throughput screening	100PL-5
200 µL	Robocolumn®: strip of 8 columns, 0.5 × 1 cm	23051508R
600 µL	Robocolumn: strip of 8 columns, 0.5 × 3 cm	23051508R-30
1 mL	Minichrom® column, 0.5 × 5 cm	23051506
5 mL (5 cm bed height)	Minichrom column, 1.13 × 5 cm	23051507
5 mL (10 cm bed height)	Minichrom column, 0.8 × 10 cm	23051504-100
10 mL	Minichrom column, 1.13 × 10 cm	23051507-100

Custom column configurations can be ordered through the OPUS PD Configurator.

Table 7. Product List: Residual Ligand Assay Kit

Description	Part Number
AVIPure HiPer Phospholipid Residual Ligand Assay Kit	9-EL-0100

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