Technical
User Guide

LentiHERO® 1
Product Code: LH0001001A

LentiHERO® 10
Product Code: LH0010001B

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PURITY by DESIGN

IMPORTANT NOTES

- Read this technical user guide before using the capsule.
- The Lenti**HERO**® chromatography capsule contains a storage buffer of 20% ethanol. For long term storage the capsule should be refrigerated between 2ºC (36ºF) and 8ºC (46ºF) and kept away from direct sunlight. For short term storage, the capsule can be kept at room temperature. Keep caps on the capsule until use. Do not freeze.
- This product is provided as non-sterile, unless specified.
- This product is sanitizable, please follow instruction included.
- This product is intended for single batch use. A clean-in-place method is provided. As feedstocks and processes vary, it is recommended that the performance of this product is validated after clean-in-place.
- The technical information can change without notice. For the latest information please refer to the Astrea Bioseparations website (https://www.astreabioseparations.com/) for the most current version of this document.

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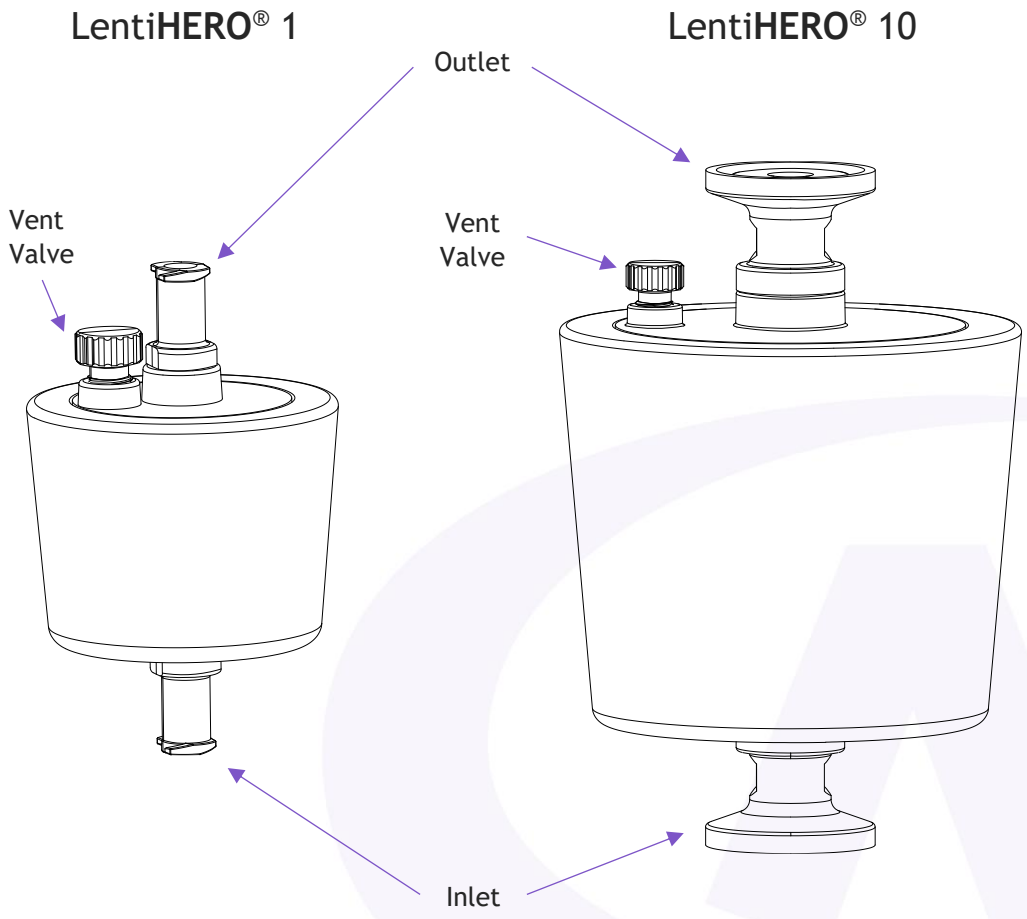
INTRODUCTION

Lenti**HERO**® is a radial pre-packed chromatography capsule designed for downstream processing of lentiviral vectors from cell culture feedstock.

Lenti**HERO**® consists of a proprietary high-flow and high-capacity composite nanofiber, Astre**Adept**®, housed in a protective shell specifically designed to reduce void volume.

The combined characteristic of a large flow path diameter, weak anion exchange chemistry and mild elution conditions enable high recovery of purified lentiviral particles.

PRODUCT DESCRIPTION



TECHNICAL DATA

SPECIFICATIONS	LentiHERO® 1	LentiHERO® 10
LIGAND:	Weak anion exchange	
TYPICAL DYNAMIC BINDING CAPACITY: Bovine serum albumin at 1mg/mL in 10mM at Tris pH 8	>60 mg/mL	
MAXIMUM PRESSURE AT 20ºC:	6 Bar | 0.6 MPa | 87 psi	
BED VOLUME (BV):	1 mL	10 mL
NOMINAL VOID VOLUME:	3.5 mL	30 mL
RECOMMENDED OPERATIONAL FLOW RATE (mL/min):	5	50
RECOMMENDED PRE-CAPSULE PRESSURE:	3 Bar | 0.3 MPa | 43 psi	
CHEMICAL STABILITY:	Compatible with buffers commonly used in bioprocessing, including 20% EtOH, 1 M acetic acid, 1 M NaOH, and solutions containing up to 10 mM EDTA	
CONNECTIONS:	Luer	Sanitary flange (12.7 mm)
VENT VALVE:	Screw cap	Screw cap
REPEATED USE:	Single batch use	
MATERIALS OF CONSTRUCTION:	HOUSING: Polypropylene, Silicone FUNCTIONAL MEMBRANE: modified cellulose acetate	

INSTALLATION

WARNING

Do not use high concentrations of organic solvents for example greater than 20% EtOH, or 30% isopropyl alcohol.

Wear appropriate personal protective equipment during operation.

Deviation from recommended guidelines could result in personal harm, or damage to the product or material.

Buffer compositions are listed in the Operations section.

Use with a liquid chromatography system

1. Using an appropriate stand (e.g., ELS-UCS-SMALL for Lenti**HERO**® 1) securely attach Lenti**HERO**® with the vent outlet facing upwards. Attach the Lenti**HERO**® to the chromatography system with appropriate tubing. For example, for Lenti**HERO**® 1 use green PEEK, 1/16" OD x 0.75mm" ID, and finger tight connectors with the supplied luer connectors.

2. Attach the column outlet position from the chromatography system to the bottom inlet of the Lenti**HERO**®, and the column inlet to the top outlet of the Lenti**HERO**® and loosen the stopper on the vent.

3. Run water through the system and once liquid is visibly exiting the vent, close the stopper on the bleed valve. Continue to run 5 bed volume (BV) of water through the Lenti**HERO**® to remove the storage buffer.

4. If required, run 5 BV of the Sanitizing Solution at 1 BV/min, and then hold for 30 minutes.

5. Run 20 BV of the High Conductivity Solution through the Lenti**HERO**® to charge the unit.

6. Equilibrate with 10 BV of the low conductivity Equilibration Buffer at 5 BV/min, until pH and conductivity are stable.

7. The system is now ready to load clarified feed that has been treated with endonuclease.

Use with a peristaltic pump system

1. Place the peristaltic pump in a clean and stable location.

2. Attach the appropriate tubing into the pump head as per manufacturers recommendations. Ensure the tubing fits securely and without kinks.

3. Test the flow rate of the tubing by priming the pump with 20% EtOH or Sanitizing Solution. Collect the liquid from the outlet into a clean tared container over 60s and record the volume. Adjust the rpm of the pump and retest until the desired flowrate is achieved (1 BV/min).

4. Attach tubing to the bottom inlet of the Lenti**HERO**®, and appropriate tubing to the top outlet of the Lenti**HERO**®. Ensure the tubing leaving the Lenti**HERO**® can connect with appropriate collection tubes or waste collection vessel. Loosen the stopper on the vent.

5. Run water through the system and once liquid is visibly exiting the vent, close the stopper on the bleed valve. Continue to run BV of water through the Lenti**HERO**® to remove the storage buffer.

6. If required, run 5 BV of the Sanitizing solution at 1 BV/min, and then hold for 30 minutes.

7. Run 10 BV of the High Conductivity Solution through the Lenti**HERO**® to charge the unit.

8. Equilibrate with 10 BV of the low conductivity Equilibration Buffer at 5 BV/min, until pH and conductivity are stable.

9. The Lenti**HERO**® is now ready to load clarified feed that has been treated with endonuclease.

Manual use with a syringe (Lenti**HERO**® 1)

1. Attach a 20 mL syringe filled with water to the bottom inlet of the Lenti**HERO**® 1. Attach appropriate tubing to the top outlet of the Lenti**HERO**® 1. Ensure the tubing leaving the Lenti**HERO**® 1 can connect with appropriate collection tubes or waste collection vessel. Loosen the stopper on the vent.

2. Once liquid is visibly exiting the vent, close the luer connector on the bleed valve. Run 20 mL of water through the Lenti**HERO**® 1 to remove the storage buffer.

3. If required, run 20 mL of the Sanitizing Solution through the Lenti**HERO**® 1, and then hold for 30 minutes.

4. Run 20 mL of the high Conductivity Solution through the Lenti**HERO**® 1 to charge the unit.

5. Equilibrate with 2 x 20 mL of the low conductivity Equilibration Buffer, until pH and conductivity are stable.

6. The Lenti**HERO**® is now ready to load clarified feed that has been treated with endonuclease.

OPERATION

Lentiviral feedstocks vary considerably in production method, lentiviral vector titer, genetic payload and contamination profile. All these factors can influence the performance of a chromatography step. A general process is described below as an example. Optimisation for use with any feedstock may be required.

EXAMPLE PURIFICATION PROCESS FOR LENTIVIRAL VECTORS

Buffers

Buffer	Composition
SANITISING SOLUTION:	0.5 M NaOH
HIGH CONDUCTIVITY SOLUTION /STRIP:	20mM Tris, 2M NaCl, pH 7
EQUILIBRATION:	20 mM Tris, 20 mM $MgCl_2$, pH 7
ELUTION:	20 mM Tris, 20 mM $MgCl_2$, 0.6 M NaCl, pH 7

Preparation of lentivirus feed

All lentivirus feeds should be treated with Endonuclease before clarification.

Lentiviral expression system	Clarification
Adherent	Centrifugation and 0.45 µM filtration
Suspension	1. Centrifugation and 0.45 µM filtration 2. 1x buffer exchange into equilibration buffer with additional 100 mM NaCl using 100 kDa molecular weight cut-off ultrafiltration membrane.

Chromatography method

1. Install, sanitize, charge and equilibrate the Lenti**HERO**® as described above Installation section.

All steps (except where noted) are run at 5 bed volumes (BV)/min.

2. Load clarified feed to approximately 1.75E+12 physical particles of lentiviral vectors/mL of adsorbent.

3. Wash with 30 BV of Equilibration Buffer.

4. Elute with 30 BV of Elution Buffer collecting the elution peak by UV absorbance. Peak volume will be determined by hold up in the system and tubing. The elution peak should be diluted immediately 1:1 with the equilibration buffer or an appropriate media to lower overall conductivity to approximately 30 mS/cm. For example, the elution peak can be collected into an equal volume of equilibrium buffer, including any appropriate stabilising additives.

5. Wash with 30 BV of Equilibration Buffer to remove the $MgCl_2$ prior to any CIP or sanitization step after use, if re-using.

6. Clean in place with 20 BV of Strip Buffer.

7. Sanitize with 5 BV of 0.5 M NaOH at 1 BV/min.

The Lenti**HERO**® can now be disposed of safely or re-charged and re-equilibrated for use with the same target.

STORAGE CONDITIONS

Store capsule between 2ºC (36ºF) and 8ºC (46ºF) away from direct sunlight. Keep caps on the capsule until use. Do not freeze.

Lenti**HERO**® is designed for single batch use. However, if storage is necessary the Lenti**HERO**® should be cleaned, and sanitized as described above, then flushed with water before being stored in 20% EtOH at 4ºC. Do not freeze. It is recommended that Lenti**HERO**® performance is validated after storage.

TROUBLESHOOTING

Problem	Probable cause	Action
High back pressure during loading	Incomplete clarification	Follow recommended procedure for clarification (e.g., suspension LV feed, centrifuge at 1500 *g* for 5 minutes, filter with 0.45 µM, buffer exchange with 100 KDa molecular weight membrane in equilibration buffer at 100mM salt.
LVV fail to bind to the Lenti**HERO**®	Conductivity of load too high	Dilute load with equilibration buffer until conductivity is approximately 15 mS/cm
Early breakthrough of LVV	Competitive binding with other impurities	Check dsDNA contamination in feed.

QUALITY ASSURANCE

The product meets the standards as described below.

COMPLIANCE OF MATERIALS:	All wetted parts have been assessed for low toxicity compliance and biocompatibility against either USP VI <88>, ISO 10993, 21CFR Part 177 or by risk assessment.
DECLARATIONS OF SUITABILITY:	TSE/BSE free, Non-Animal derived, Nitrosamine free statements available
QUALITY:	Manufactured within an ISO 9001:2015 Quality Management System the product is controlled, traceable and released through the Quality function. For research use only.
COMPLIANCE OF PERFORMANCE AND IDENTIFICATION:	Flow test and inspection

ORDER INFORMATION

Code	Description	Pack Size
LH0001001A	Lenti**HERO**® 1	1 capsule
LH0010001B	Lenti**HERO**® 10	1 capsule

For more information on this or any other supply related matters, please do not hesitate to contact us at sales@astrea-bio.com

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Issue Date: 03 May 2024
CCR Number: N/A - additional info. available & added.
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