A Novel Purification Technology to increase Processing Efficiency, Purity and Recovery of Lentiviral Particles for Viral Vector Development

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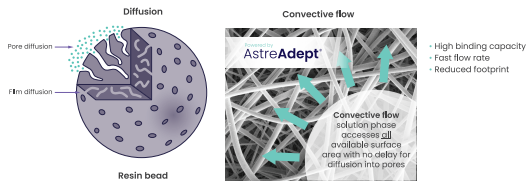
1 Introduction

Immediate access to high binding surface area enables more effective processing of lentiviral vectors

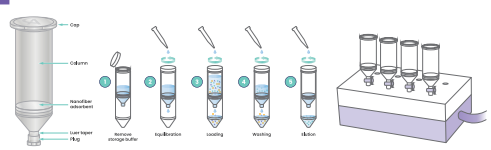


Therapeutic developers face significant challenges in purifying cell and gene therapy modalities. Current processes rely on solutions developed for monoclonal antibodies and are not fit-for-purpose for viral vector purification. Resin chromatography has significant limitations, such as reduced yield, long processing time, and expense.

AstreAdept® is a novel nanofiber material that addresses the challenges of purifying large and fragile modalities, which are often produced at low titer with high levels of contaminants.

AstreAdept® has extremely accessible fibers which allows almost immediate access to high binding surface area at high flow rates. Here, we present how this technology has been incorporated into a spin column format, the Nereus LentiHERO® to bring these advantages to viral vector purification at lab-scale.

2 Nereus LentiHERO® for Lentivirus Purification



Easy to use spin column format

Sample loading, up to 45 mL, via bench top centrifuge

Load larger volumes and multiple samples simultaneously via a vacuum manifold

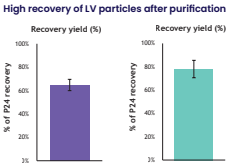
3 Significant Increases in Lentiviral Yield vs. Current Processes

High binding capacity and high recovery enables efficient use of lab-scale lentiviral processing

High LV purification capacity per spin column

LV Load :	2.17E+9 vp/mL	
Loading Volume (mL)	Total LV particle	
	Load	Elution
15	3.26E+10	1.06E+10
30	6.52E+10	4.89E+10
60	1.30E+11	4.55E+10
LV recovery 4.7E+10 particles		

High recovery of LV particles after purification



Method

Different volumes of lentivirus feedstock (produced from HEK293T cells) were loaded separately, and purified following the technical user guide (NL100100). Load and eluate samples were analysed by P24 ELISA (ZeptoMetrix) to determine physical titre of lentivirus.

- Feedstocks from 2 different external manufacturers (Feed A, Feed B) were independently purified and assayed (n=3/group).
- The total % of P24 recovery yield was relative to LV input
- 60 - 80% LV particle recovery post purification with LentiHERO®

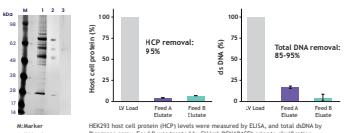
4 Nereus LentiHERO® vastly decreases host cell proteins and DNA contamination

Reducing HCP contamination even at lab-scale is important to reduce unwanted immunological effects impacting results

Purification with **Nereus** LentiHERO®

- 95% reduction in host cell protein contamination
- Host cell proteins were not detectable in the eluate, via a silver stained SDS-PAGE
- 85-95% removal of double stranded DNA after purification

Significant reductions in host cell proteins and total DNA



M:Marker
1: LV load
2: Flow-through
3: LV Eluate

HEK293 host cell protein (HCP) levels were measured by ELISA, and total dsDNA by Picogreen assay. Feed B was treated by SU/mL DENARASE® prior to clarification.
LV Load HCP concentration: Feed A, ~ 2µg/mL; Feed B, 0.8µg/mL.
LV load dsDNA concentration: Feed A, ~ 1.5µg/mL; Feed B, 0.5µg/mL.

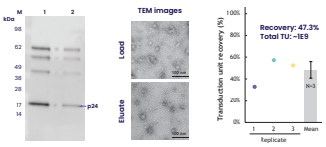
Silver stained SDS-PAGE gel

Total protein profiles were analysed using load, flow-through and elution fractions. Samples were run on SDS-PAGE gel and visualised by a silver stain.

5 Structure & Infectivity are not compromised

Purified lentiviral particles retain functionality, morphology and size

- The size of important proteins such as Gag, encoding capsid, matrix, and nucleocapsid, were unaffected
- Eluted lentiviral particle size and morphology were not affected by purification with **Nereus** LentiHERO®
- The infectivity of purified lentiviral particles was not comprised



LV specific p24 Gag protein was detected using rabbit polyclonal anti-p24 IgG by Western blot analysis. Two repeats of LV eluate were analysed as shown in Lane 1 and 2. The gag proteins such as p24 were identified around 24 kDa together with another major gag p55 around 55 kDa. This pattern was identical to that of concentrated LVs with molecular weight cut-off (100kDa) filtration method (data not shown).

M:Marker
1: LV Elute 1
2: LV Elute 2
Ab: polyclonal anti-P24 IgG

Transmission electron microscopic (TEM) images of load and eluate fractions using a negative staining method.

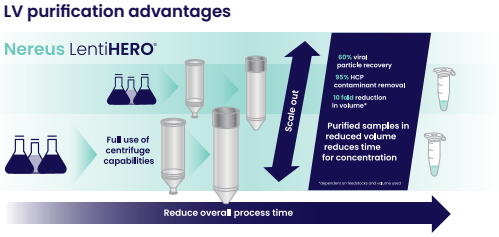
Infectivity of LVs from the purified eluates were tested by transduction assay to Jurkat cell line measured by flow cytometry

6 Combining Yield, Purity and Accelerated Processing

- Maximise sample throughput by utilising the total benchtop centrifuge capacity
- Contaminants are removed and sample volumes are quickly reduced
- Purer, reduced volume samples require shorter concentration times

	Nereus LentiHERO®	Ultracentrifugation/ density gradients	IEX resin Gravity fed	IEX Membrane Adsorbers	Concentrators
Purification	• Short purification time • Reduces 95% HCP • Fewer loses of yield	Combined purification and concentration	Low yield	Low yield	Limited purification
Concentration	• Purified samples are processed faster	• Requires training • Often not available in cell culture labs	Limited	Limited	Contaminants are concentrated along with lentiviral particles
Throughput	• Multiple samples processed simultaneously	Limited throughput	Limited throughput	Limited throughput	Limited throughput

7 LV purification advantages



Nereus LentiHERO® Summary

- Powered by AstreAdept® technology, a novel nanofiber membrane
- Ease-of-use design, enabling rapid and high recovery of functional lentiviral particles
- Significant reduction in contaminants such as host cell proteins
- Simultaneous processing of multiple samples, reducing bottlenecks for viral vector development sample preparation.

By enabling researchers to accelerate the development of cell and gene therapies, this technology ensures a more cost- and time-efficient way to speed the delivery of novel therapeutics to patients in need.

Information

Contact info: sales@astrea-bio.com
Order information: Product code NL100100, 2 unit pack

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