Technical
User Guide

Nereus LentiHERO®

Product Code: NL100100

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PURITY by DESIGN

INTRODUCTION

Instructions for use

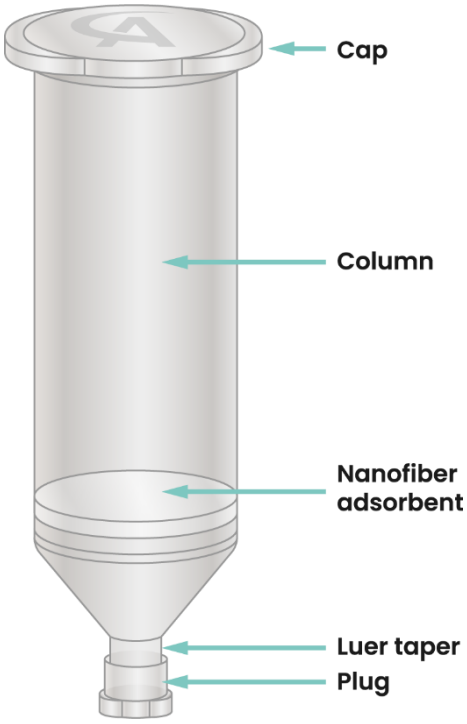
Nereus Lenti**HERO®** offers the combined benefits of very high recovery of lentiviral particles, significant reduction of contaminants and ability to scale out.

The spin column format is used with a benchtop centrifuge, with no need for ultracentrifugation, large chromatography systems, or tedious filtering by hand. Sample throughput can be increased simply by maximizing the benchtop centrifuge capacity.

With **Nereus** Lenti**HERO®** feedstock volumes are reduced in the order of 10-fold depending on loading volume. Decreasing both contaminant levels and sample volumes reduces the time required for further concentration steps if desired, allowing more samples to be concentrated in any given day.

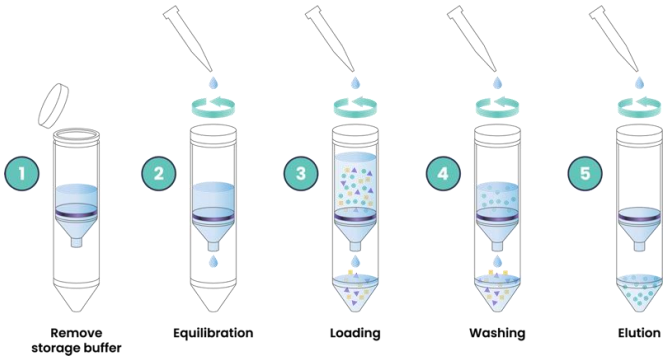
As the lentiviral feedstock production method and titer will affect the performance of the **Nereus** Lenti**HERO®**, a general protocol is given here. However, recommendations on process optimisation are also included that may allow the final processed volume to be reduced to 2 mL, or for feedstocks to be used direct from harvest.

Schematic of **Nereus** Lenti**HERO®**

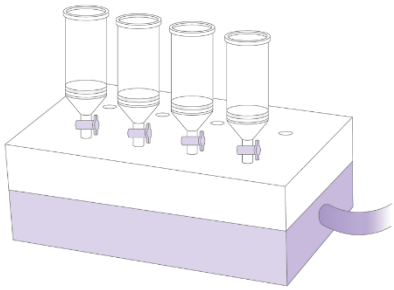


Easy to use spin column format

Protocol overview



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



Load larger volumes and multiple samples simultaneously via a vacuum manifold.

Storage conditions | Shelf life | Quality Control

The **Nereus** Lenti**HERO®** device contains a storage buffer of 20% ethanol. For long term storage the device should be refrigerated at 4˚C. For short term storage, the device can be kept at room temperature. It is important that these devices should not be allowed to dry out, as performance could be affected.

The **Nereus** Lenti**HERO®** should be used within one year of purchase.

This product has passed a flow test and an inspection test.

Working with recombinant lentivirus

To avoid the potential hazards associated with recombinant lentivirus, it is recommended that precautions and practices established in Biosafety Level 2 guidelines are followed when using this device.

Equipment required per purification

50mL Centrifuge tubes with filter caps, for example Z761028-180EA Sigma Aldrich

1x **Nereus** Lenti**HERO®**

Centrifuge capable of 1,000 x g spin speed, preferably refrigeration to 4 ˚C

The maximum volume that the column can receive depends on the centrifuge used. For a swing-out centrifuge the maximum volume is up to 15 mL. In a fixed angle centrifuge, this is limited to 12mL for optimal performance.

Nereus Lenti**HERO®**
GENERAL PURIFICATION PROTOCOL

Buffers

Buffer	Composition	pH
Wash Buffer	20 mM Tris 20 mM $MgCl_2$	pH 7
Elution Buffer	20 mM Tris 20 mM $MgCl_2$ 600 mM NaCl	pH 7
Prime Buffer	1M NaCl	pH 7

If you generally use buffers with additives that have been shown to minimise lentiviral vector binding to plastics or tubing, then we would recommend using those for the equilibration and wash steps; and adding sodium chloride to 600 mM to make the elution buffer.

Method

Please note:

- Read the entire protocol before starting.
- The use of frozen-thawed feedstock will negatively impact lentiviral vector recovery. It is advisable to use fresh feedstocks where possible.
- The recommended maximum capacity per device is 2E+10 viral particles.
- Feedstocks should be clarified via centrifugation and a 0.45 µm filter prior to processing with the **Nereus** Lenti**HERO®**.
- The presence of high salt in harvested media is detrimental to lentiviral quality and will also prevent capture of lentiviral particles.
- Collect and save the flow through for later analysis, if you need to investigate the recovery of lentivirus at each step.
- Centrifuge spin times are dependent on the individual feedstocks used. Extend the duration of the centrifuge spin if the feedstock does not pass through the filter in the recommended time. **Do not increase the speed of rotation.**
- This method is based on the use of 50 mL tubes with filtered caps. If solid caps are used, the spin times may need to be extended. **Do not increase the speed of rotation.**
- This product is for research use only.

Preparation of spin column

1. Place a rack inside the tissue culture hood. Remove the **Nereus** Lenti**HERO®** from the packaging, and place inside the tissue culture hood. Remove the cap and discard the 20% EtOH storage buffer by tipping it out into a suitable waste container. Remove the plug.

2. Place the **Nereus** Lenti**HERO®** into a clean 50mL tube. Fit the filter lid onto the tube. Centrifuge at 1,000 x g for 2 min. Return the **Nereus** Lenti**HERO®** to the rack. Lift out the **Nereus** Lenti**HERO®** from the 50 mL tube and discard the flowthrough. Replace the spin column into the tube.

3. Optional: the device can be sanitized with 4mL of 500 mM NaOH (centrifuge at 1,000 x g for 2 min). Discard the flowthrough.

4. Pipet 4 mL Prime Buffer into the column, making sure to wash the inside of the column to remove residual ethanol. Centrifuge at 1,000 x g for 2 min. Return the **Nereus** Lenti**HERO®** to the rack, and discard the flowthrough.

5. Pipet 4 mL Wash Buffer into the column. Centrifuge at 1,000 x g for 2 min. Return the **Nereus** Lenti**HERO®** to the rack, and discard the flowthrough.

Loading and wash of sample

Clarified lentiviral feedstock can be loaded on to the **Nereus** Lenti**HERO®** in several ways depending on the volume being processed.

For volumes 5-45 mL, the device can be loaded multiple times to process your sample up to 45 mL:

6. Load your sample, (up to 15 mL in a swing out centrifuge, 12mL in a fixed angle centrifuge) into column of the **Nereus** Lenti**HERO®**. Centrifuge at 1,000 x g for 2 min, at 4 °C.

7. Repeat the loading step, until the load is complete, discarding the flow though after every spin and using a clean centrifuge tube for each aliquot loaded.

8. When sample loading is complete, add 4 mL Wash Buffer to the spin column, rinsing the buffer down the sides of the column to ensure all the feedstock is washed down to the filter. Centrifuge at 1,000 x g for 2 min, at 4 °C.

9. Proceed to elution step.

For volumes greater than 45 mL:

10. Samples can be loaded by attaching multiple **Nereus** Lenti**HERO®** devices to a vacuum manifold. Alternatively, samples can be drawn through the **Nereus** Lenti**HERO®** column by attaching a tubing and peristaltic pump system to the luer taper.

    Flow rates should be adjusted for a maximum rate of 2 mL/min. When sample loading is complete, add 4 mL Wash Buffer to the column, rinsing the buffer down the sides of the column to ensure all media is washed down to the filter. Draw the buffer through the nanofiber adsorbent with the chosen method.

11. Proceed to elution step.

Elution of retained Lentiviral vectors

Regardless of which of the above methods was used for the loading and washing steps, the elution proceeds with the same following steps:

12. Add 4 mL Wash Buffer to a clean collection 50 mL tube. This will receive the purified lentiviral sample, allowing the salt containing elution buffer to be immediately diluted to preserve lentiviral vector functionality.

13. Place the **Nereus** Lenti**HERO®** device into this collection tube and pipette 4 mL Elution Buffer into the column. Proceed without delay to centrifuge the device at 1,000 x g for 2 minutes, at 4 °C. This flow through is your purified sample.

14. The purified lentiviral vector sample is in a final volume of 8mL. This volume can be reduced through process optimization, see recommendations below. If required, the purified sample can then be further concentrated using either:

    a. A molecular weight 100 kDa cut-off filter
    b. Precipitation via a standard PEGylation method

15. Titrate the virus immediately and/or store at -80°C for longer term storage.

Process Optimisation

The **Nereus** Lenti**HERO**® performance is affected by lentiviral feedstock production methods and titer. Once you are familiar with the basic protocol, we recommend optimizing the protocol for your particular feedstock.

Process step	Optimization
Feedstock preparation	The basic protocol uses a feedstock that is clarified via a brief centrifugation, and filtration through a 0.45 µm filter. Depending on the particular feedstock used, it may be possible to remove the filtration step, or even the clarification centrifugation step and load direct from harvest. Please note that if the filtration or centrifugation steps are removed, the centrifugation duration may need to be extended. The centrifugation speed should not exceed 1000 x g.
Elution volume	The final sample volume can be reduced by using smaller volumes of Elution and Wash buffer in the Elution step, using a 1:1 ration of these 2 buffers. For example, using 1 mL Elution Buffer and 1 mL Wash Buffer will result in a final sample volume of 2 mL. The optimal volumes used in the elution step will depend on the lentiviral feedstock used.
Centrifugation temperature	It may be preferable to maintain a stable sample temperature. In this case, the centrifuge temperature should be adapted to match the feedstock sample.

ORDER INFORMATION

Code	Description	Pack Size
NL100100	**Nereus** Lenti**HERO®**	2 unit pack

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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