Technical
User Guide

pDNAHERO® 100

Product Code: PHP0100001C

Powered by AstreAdept®

Search: Astrea Bioseparations

Astrea Bioseparations

PURITY by DESIGN

IMPORTANT NOTES

- Read this technical user guide before using the device.
- pDNA**HERO**® devices contain a storage buffer of 20% ethanol. For long term storage, the device should be refrigerated between 2 ºC (36 ºF) and 8 ºC (46 ºF) and kept away from direct sunlight. For short term storage, the device can be kept at room temperature. Keep caps on the device until use. Do not freeze.
- This product is provided as sanitized, unless specified.
- This product is intended for single batch use. A clean-in-place (CIP) method is provided. As feedstocks and processes vary, it is recommended that the performance of this product is validated after CIP.
- This 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.

TABLE OF CONTENTS

INTRODUCTION **3**

PRODUCT DESCRIPTION **4**

TECHNICAL DATA **5**

INSTALLATION **6**

Use with FPLC system *6*

Preparation of feed *7*

*Chromatography method for pDNA**HERO**®* *9*

Buffers *9*

STORAGE CONDITIONS **11**

TROUBLESHOOTING **12**

QUALITY ASSURANCE **13**

ORDER INFORMATION **13**

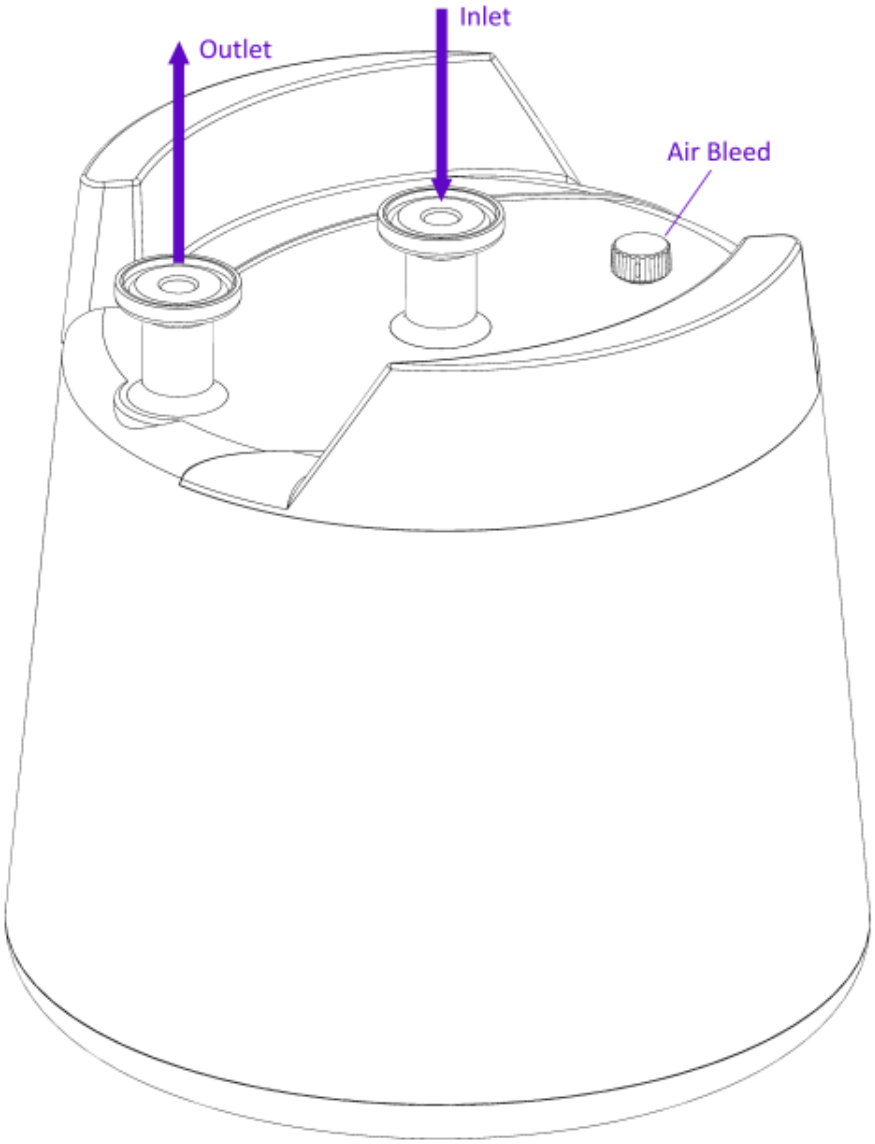
INTRODUCTION

pDNA**HERO**® is a radial pre-packed chromatography device designed for downstream processing of plasmid DNA.

pDNA**HERO**® consists of a proprietary high-flow and high-capacity composite nanofiber, Astre**Adept**®, housed in a protective shell specifically designed for fast flow rates.

The combined characteristic of a large flowpath diameter, hydrophobic interaction ligand, and mild elution conditions enable high recovery of purified plasmid DNA.

PRODUCT DESCRIPTION



TECHNICAL DATA

SPECIFICATIONS	pDNA**HERO**® 100
LIGAND:	Hydrophobic interaction chromatography
TYPICAL DYNAMIC BINDING CAPACITY: Herring Sperm DNA	> 6 mg/mL
MAXIMUM PRESSURE AT 20 **°C:**	6 Bar | 0.6 MPa | 87 psi
BED VOLUME (BV):	100 mL
NOMINAL VOID VOLUME:	300 mL
RECOMMENDED OPERATIONAL FLOW RATE (mL/min) [minimum to maximum range]:	500 [50 mL/min–1000 mL/min]
RECOMMENDED PRE-DEVICE PRESSURE:	0–3 Bar | 0–0.3 MPa | 0–43 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:	Sanitary flange (12.7 mm)
VENT VALVE:	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.

Use with FPLC system

1. Attach the pDNA**HERO**® device to the chromatography system with 12.7 mm TC connectors and appropriate size tubing (for example, PTFE/silicone tubing 9.5 mm ID).

2. Attach the column outlet from the chromatography system to the inlet of the device (center connector), and the column inlet from the chromatography to the outlet of the device (outer connector) and loosen the stopper on the bleed valve.

3. If required, run a sanitizing solution (e.g., 0.5 M NaOH), at 1 bed volume (BV)/minute for 30 minutes.

4. Once liquid is visibly exiting the bleed valve, close with the stopper. Continue running until the pH of the FPLC outlet is stable at the same pH as the sanitizing solution.

5. Wash the device with water until the conductivity is below 10 mS/cm.

6. Equilibrate the device with the desired buffer (e.g., Tris 50 mM, 10 mM EDTA, 3 M ammonium sulphate pH 8) at 1 BV/minute until pH and conductivity are stable.

7. Gradually increase flow up to 5 BV/minute. Ensure that pre-column pressure does not go higher than 3 bar. The pressure should be between 0.1–2 bar.

   The system is now ready to load nucleic acid feed conditioned to between 2.85–3 M ammonium sulphate preferably with 10 mM TE.

OPERATION

Preparation of feed

pDNA clarification will vary according to the process. An example of an alkaline lysis procedure to process pDNA expressed in *E. coli* culture is outlined below. This method can be used for larger plasmid lysate preparations to reduce buffer volumes and accommodate larger-scale mixing.

EXAMPLE OF AN ALKALINE LYSIS PROCEDURE

1. Weigh out cell paste from centrifugation of *E. coli* culture, and let it thaw to room temperature or until no longer icy.

2. Add 10x volume of STES buffer (8% Sucrose, 50 mM TRIS, 100 mM EDTA, 2% SDS pH 8.0) to the weight of the cell paste, i.e., 10 mL buffer per 1 g of paste.

3. Mix for 30 minutes with an overhead stirrer until cells have been well suspended (duration may vary depending on scale).

4. Continue stirring and add 5 M NaOH dropwise until the pH has reached 12.0–12.2. At larger scales, a pump is recommended. Slow the rate of addition when reaching > 11 pH because the material will become extremely viscous, and mixing is not uniform. Do not allow the pH to go over 12.4 as it will begin to denature the plasmid DNA.

5. Once at pH 12.0, let the cell lysate mix for ~30 minutes to ensure full lysis and denaturation of all the cells and gDNA.

6. Neutralize this mixture with 3 M potassium acetate pH 5.5, adding it in dropwise while mixing to bring the pH down to 8.0. Plasmid DNA is quite stable, so if the pH drops to 7.0 or below, the pH can be adjusted back up as required for capture.

7. Mix for 20–30 minutes until the pH is stable and viscosity is reduced.

8. Centrifuge for 30 minutes at 10 000 x *g* at 4 ºC.

9. Collect the supernatant.

10. Add 5 M $CaCl_2$ in dropwise until a concentration of 0.7–1 M $CaCl_2$ is reached in the lysate. Mix for 20 minutes at room temperature. Turbidity may be observed at this point. Some adjustment to the $CaCl_2$ amount may be needed to accommodate the plasmid sequence.

11. Centrifuge 20 minutes at 12 000 x *g* at 4 ºC.

12. Collect the supernatant. This is $CaCl_2$ clarified lysate, which should be plasmid DNA enriched relative to the RNA content of the original lysate.

13. Buffer exchange to the equilibration buffer of the chromatography primary capture step with an appropriate molecular weight cut-off membrane or dilute to the required conductivity (35–36 mS/cm) with 50 mM Tris, 5 mM EDTA.

EXAMPLE OF PRIMARY CAPTURE OF PDNA POST-ALKALINE LYSIS

DEAE PuraBead® HF resin (Product code 3451) is a recommended adsorbent for primary capture and is available in pre-packed columns or as loose resin.

1. Columns packed with DEAE PuraBead® HF resin can be sanitized with 1 M NaOH at 0.3 column volumes (CV)/minute for 10 CV.

2. Equilibrate the column with 50 mM Tris, 5 mM EDTA, at 0.5 CV/minute for 10 CV.

3. Charge the column with 50 mM Tris, 5 mM EDTA, 2 M NaCl, at 1 CV/minute for 10 CV. Re-equilibrate the column with 50 mM Tris, 5 mM EDTA, at 1 CV/minute for 5 CV. The column is now ready to load with post-$CaCl_2$ clarified lysate conditioned to 35–36 mS/cm (load up to 1 mg of pDNA per mL of resin), load at 0.5 CV/minute.

4. Wash the column with 50 mM Tris, 5 mM EDTA, at 1 CV/minute for 5 CV.

5. Run a gradient elution to 100% 50 mM Tris, 5 mM EDTA, 1 M NaCl over 20 CV running at 1 CV/minute to establish where the target elutes. Collect peaks by UV absorbance.

6. Strip the column with 50 mM Tris, 5 mM EDTA, 2 M NaCl, at 1 CV/minute for 5 CV.

The column is now ready to be cleaned or sanitized in place with 1 M NaOH at 0.3 CV/minute for 10 CV. This step and subsequent equilibration can be run in reverse where possible to increase the efficiency of the cleaning process.

Once cleaned, wash the column with 20 CV RO (Reverse Osmosis) water, and then store in 20% EtOH.

Chromatography method for pDNA**HERO**®

Production methods vary considerably, and many different factors can influence the performance of a chromatography step. A general process is described below as an example. **Optimization for use with any feedstock may be required.**

EXAMPLES OF PREPARATION OF ELUTION POOLS FROM PRIMARY CAPTURE

The elution pool from primary capture should be conditioned for binding to give a final concentration of 2.85–3 M ammonium sulphate for the load. For example, carry out a dilution with at 1 volume of feed to 20 volumes of 3 M ammonium sulphate to give a concentration of 2.85 M ammonium sulphate in the feed.

For clarified lysate with RNA removed either by RNAse treatment and size exclusion chromatography, calcium chloride precipitation, or diafiltration:

Start with a 1:1 dilution with 4 M ammonium sulphate and perform clarification of any precipitation by centrifugation or 0.8 µM filtration. Finally, carry out a further 1:1 dilution of that clarified material with 4 M ammonium sulphate to give a 3 M ammonium sulphate concentration.

In all cases, pre-chromatography clarification with 0.8 µM filters is recommended.

Buffers

Buffer	Composition
SANITIZING SOLUTION:	0.5 M NaOH
EQUILIBRATION:	3 M ammonium sulphate in Tris 50 mM, 10 mM EDTA, pH 8
ELUTION:	Tris 50 mM, 10 mM EDTA, pH 8
STRIP:	Deionized water

1. Install, sanitize, and equilibrate the column as described above installation Section 4.

2. All steps (except where noted) are run at 5 bed volumes (BV)/minute (500 mL/min).

3. Equilibrate with 5 BV of equilibration buffer.

4. Load clarified feed. Capacity will vary according to sequence length and composition. Loading ~2.5–3 mg of pDNA per mL of adsorbent is average for sequences under 10 kb. Sequences up to 35 kb have been purified using pDNA**HERO**®.

5. Wash with 30 BV of equilibration buffer.

6. Elute with a gradient to 100% elution buffer over 30 BV of elution buffer collecting the elution peak by UV absorbance, to establish where the target elutes. This can be used to establish a step wise gradient to elute the pDNA. For example:

    a. Elute with 20 BV of 10% elution buffer.

b. Elute with 20 BV of 80% elution buffer; Note that 75–80% of the pDNA between 5–34 kb elutes in this step.

c. Elute with 20 MVs of 100% elution buffer.

7. Clean in place with 10 BV of Strip buffer (deionized water).

8. Sanitize with 10 BV of 0.5 M NaOH at 1 BV/minute (100 mL/min). This step and subsequent wash and re-equilibration can be run in reverse flow to increase efficiency of column cleaning.

9. Wash to low conductivity with Strip buffer (water).

10. Re-equilibrate with 10 BV of equilibration buffer.

The device can now be disposed of safely or re-equilibrated for immediate use.

STORAGE CONDITIONS

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

pDNA**HERO**® devices are designed for single batch use. However, if storage is necessary, the device should be cleaned as described in "Chromatography method for pDNA**HERO**®" section and stored in 20% EtOH at 4 ºC. Do not freeze. It is recommended that pDNA**HERO**® device performance is verified after storage.

TROUBLESHOOTING

Problem	Probable cause	Action
Delta column pressure increases during loading	Incomplete clarification	Filter the load through a 0.8 µM filter
pDNA fails to bind to pDNA**HERO**®	Insufficient concentration of kosmotropic salt in the load	Add more ammonium sulphate to achieve a concentration of 3 M
Increase in delta column pressure between runs	Incomplete removal of tightly bound impurities	Running at 100 mL/min, wash the unit with 2 L of water, fill with 1 M NaOH and leave overnight the unit with no flow. Wash the unit with 20 BV of water and re-equilibrate
Early breakthrough of pDNA	Competitive binding with other impurities	Check concentration of RNA or other competing impurities. Screen calcium chloride (0.8–1 M) precipitation, or diafiltration before primary capture to remove more RNA

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, GMO and Country of Origin available within the associated Process-ready Regulatory Support File
QUALITY:	Manufactured within an ISO 9001:2015 Quality Management System the product is controlled and traceable. Manufactured within an ISO 8 environment before being released through the Quality function
COMPLIANCE OF PERFORMANCE AND IDENTIFICATION:	Flow test, visual inspection, bioburden, and endotoxin

For further details, refer to the associated Process-ready Regulatory Support File.

ORDER INFORMATION

Code	Description	Pack size
PHP0100001C	pDNA**HERO**® 100	1 device

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

+44 (0) 1223 433 800 | astreabioseparations.com

sales@astrea-bio.com | techsupport@astrea-bio.com | quality@astrea-bio.com

Global bases in North America, Canada and Cambridge UK HQ:
Horizon Park, Barton Road, Comberton, Cambridge, CB23 7AJ, UK

Issue Date: 18 Nov 2024
CCR Number: N/A - NPI
Author Name: D Steel
QA Reviewer Name: R Hawkins

Astrea Bioseparations