Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Data Sheet

BioContinuum™ Seed Train Platform

Mobius® High Cell Density Cryopreservation Assemblies

Increase Your Cell Expansion with Lower Risk of Contamination and Improved Reproducibility

Our Mobius® High Cell Density Cryopreservation (HCDC) assemblies enable seed train inoculation with cells at a higher cell density and larger volume compared to the traditional vial-based seed train process. This higher volume eliminates multiple vial expansion steps to significantly accelerate the cell expansion process. These assemblies are specifically designed to facilitate the freeze and thaw of cryopreserved cells in either ultra-low temperature (-80 °C format) or liquid nitrogen (-196 °C format) freezers. Both formats are available in two assembly bag sizes of 100 mL and 250 mL in a 5-bag manifold. This multi-bag design significantly reduces environmental contamination risk compared to vials or single bag operations, and improves process reproducibility by providing uniform inoculation feed across multiple campaigns.

Benefits

- Time & cost savings by elimination of expansion steps
- Reduced risk of contamination with closed process
- Improved reproducibility
- Improved operational throughput/flexibility

MERCK

Streamline Cell Expansion with High Cell Density and High Volume

The traditional cell expansion process for a batch starts with thawing a single vial of cells and can take up to 30 days before inoculation of the production bioreactor. The Mobius® HCDC assembly with bag sizes of 100 mL or 250 mL allows cells to be frozen at either -80 °C, or liquid nitrogen vapor phase temperature down to -196 °C, at higher cell density (> 50 x 10^6 cells/mL) and larger volume using the HCDC method. This streamlined method of initiating cell expansion will reduce the number of intermediate operation steps, allowing biomanufacturers to shorten the expansion process by 10 days due to the elimination of multiple expansion steps.

Table 1 highlights use of HCDC method for both a 15,000 L fed-batch and a 2,000 L perfusion bioreactor. In the examples shown, a 250 mL bag (with 150 mL working volume) of 70 x 10^6 cells/mL provide a Viable Cell Count (VC) of 10,500 x 10^6. This is a sufficient quantity to enable seeding to commence at N-3 (fed-batch) and N-2 (perfusion) compared to traditional expansion started with a 1 mL vial of 10 x 10^6 cells/mL.

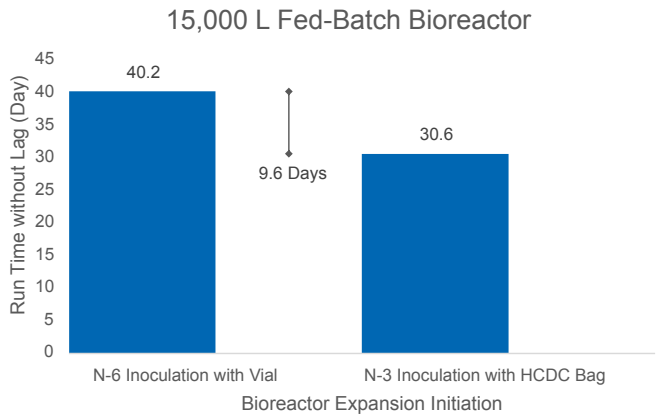
Closed Design Reduces Risk of Contamination

In contrast to the open cell culture operation required for the conventional cell expansion method using vials, the HCDC process employs a closed assembly design which significantly reduces the risk of contamination and improves reproducibility (See **Figure 1**).
The Mobius® HCDC assembly design consists of five cryobags, a 1 L waste bag for flushing lines, and tubing with clamps and metal NovaSeal pinch pipes.
Each cryobag can be securely sealed and disconnected using the NovaSeptum® crimping tool, ensuring a closed and sterile environment for your cell culture.

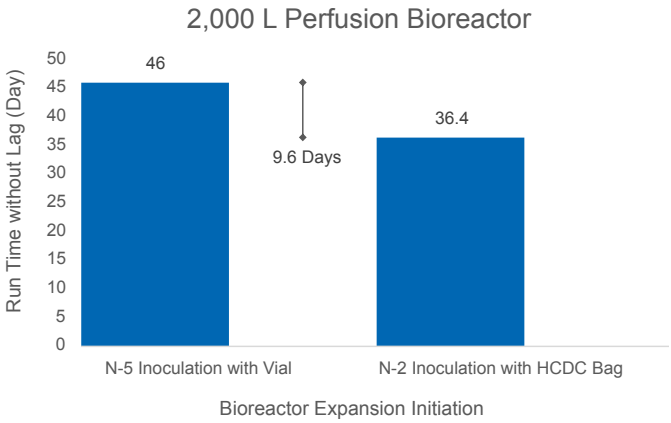
Solution for Production Seed Train Intensification

The incorporation of high volume, high cell density cell banking into the seed train process can enable manufacturers to decouple early seed train operations from bioreactor inoculation steps of the process. This closed process operation saves time in the manufacturing facility and enables reduced room classification. While it may be possible to leverage larger vials for this process, the amount of cells required to eliminate inoculation steps require larger volumes of 50–250 mL cryobags.

Table 1. Comparison of process using inoculation with a vial versus a cryobag. Use of high cell density cryopreserved cells significantly reduces process time.



Fed-Batch Bioreactor	Volume (L)	Inoculation Cell Count (x 10E6)	Run Time (Day)
N (Production Bioreactor)	15,000	7,500,000	21
N-1	1,500	750,000	24.2
N-2	150	75,000	27.4
N-3	15	7,500	30.6
N-4	1.5	750	33.8
N-5	0.15	75	37
N-6	0.015	7.5	40.2



Perfusion Bioreactor	Volume (L)	Inoculation Cell Count (x 10E6)	Run Time (Day)
N (Production Bioreactor)	2,000	1,000,000	30
N-1	200	100,000	33.2
N-2	20	10,000	36.4
N-3	2	1,000	39.6
N-4	0.2	100	42.8
N-5	0.02	10	46

Solution for Process Development

The HCDC method also improves batch reproducibility compared to the traditional seed train method. Using HCDC, cells produced in one seed train expansion step can be stored to inoculate multiple bioreactors, ensuring an equal starting point for different bioreactor runs.

Supported by the Emprove® Program – The Smart Way to Master Compliance and Control

Complementing our product portfolio, the Emprove® Program provides convenient access to reliable technical, regulatory and supply information in Emprove® Dossiers to support your risk assessment continuum. A subscription to our Emprove® Suite can help you stay current: In addition to accessing the Emprove® Dossiers, you can also receive notification updates to document changes, as well as generate metrics and reports.

For more information, please visit:
SigmaAldrich.com/emprove

Solution for Design of Experiments (DOE) of Bioreactor Process Conditions

When conducting experiments at different time points, HCDC also removes the need of repeated cell expansions which may yield variable expansion results. As even small changes in the expansion process can impact the cell growth rate, the benefit of equivalent starting points provided by the HCDC method eliminates a major variable in time consuming Design of Experiments (DoE) of bioreactor process conditions. This saves time and cost by reducing the number of control bioreactors needed.

HCDC Process Made Easy

Filling and inoculation using the Mobius® HCDC assembly is straightforward and safe. In the example shown in **Figure 2** using a 250 mL bag assembly, the HCDC process begins by filling a 250 mL cryobag with 50 mL of the cryopreservation medium (including DMSO) and then with 100 mL of the cell suspension[1].

Once a cryobag is filled, it is easily disconnected via the NovaSeptum® crimping tool, placed in a protective case[2] and stored in the freezer at -80 °C. For inoculation, the cryobag is removed from the freezer, thawed in a water bath, and connected to the seed train bioreactor.

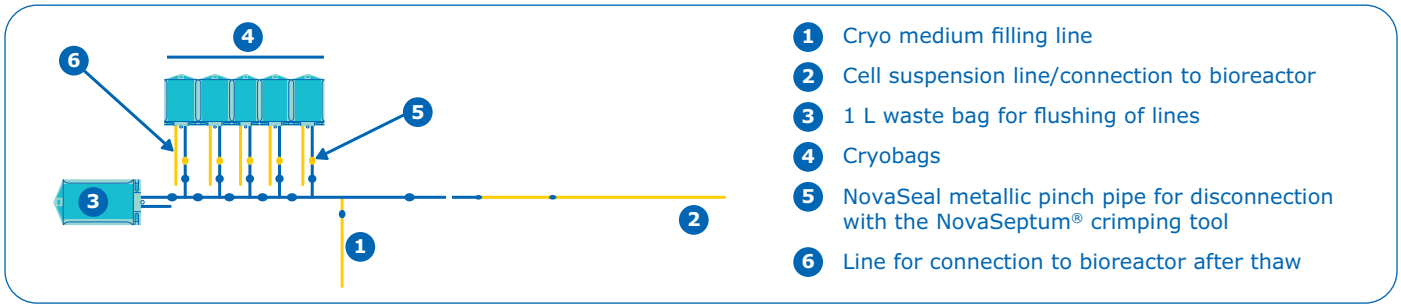


Figure 1. Components of a Mobius® HCDC Assembly

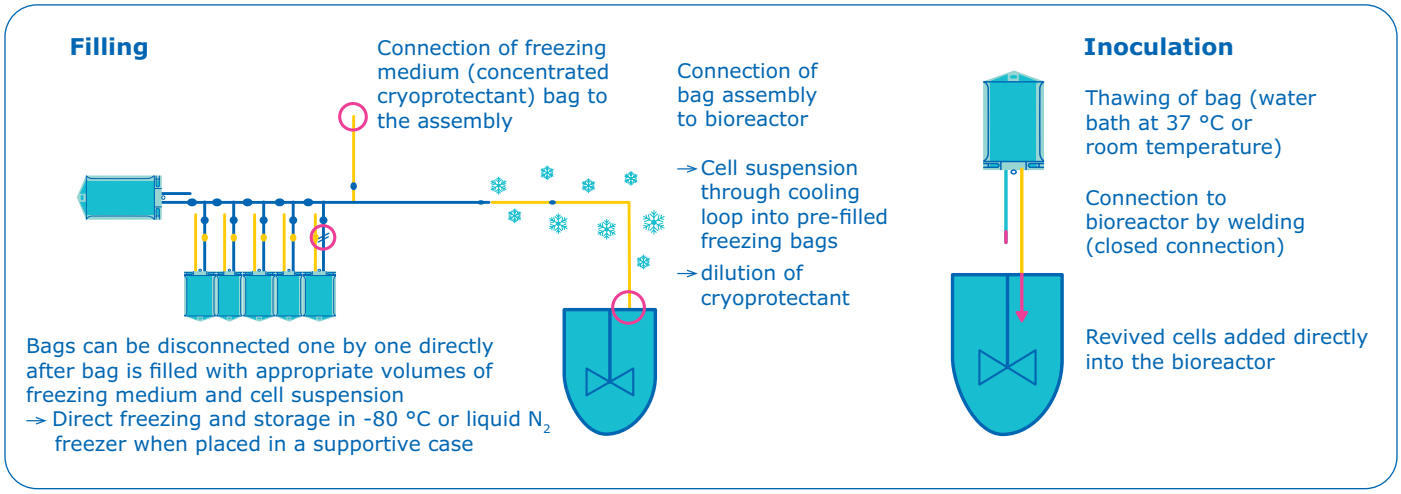


Figure 2. Filling and inoculation process using a Mobius® HCDC Assembly

References

1. For filling of the cryobag, we recommend the combination of cell suspension and cryopreservation medium does not exceed 70% of the overall cryobag volume.
2. To ensure the integrity of the cryobag, we recommend placing the filled cryobag in a protective case for the entirety of the freezing and thawing process. Handle the cryobag with care at all times.

Part of the BioContinuum™ Seed Train Platform

There are multiple ways to intensify your seed train process. Whether you are adopting the HCDC approach to accelerate cell expansion or using N-1 perfusion to achieve greater cell mass, our integrated seed train platform has specially designed products to realize your seed train intensification goal.

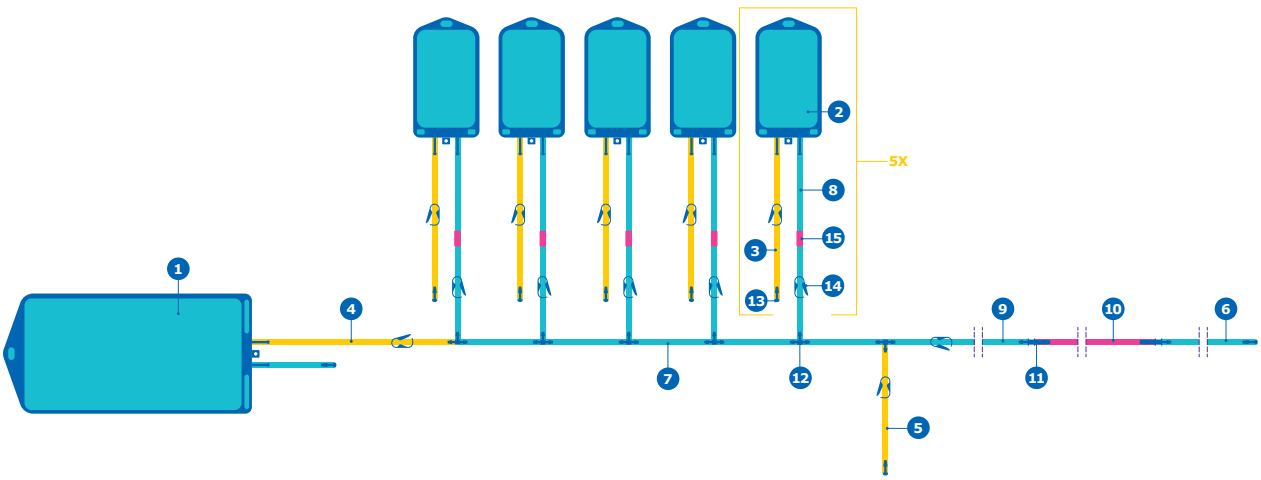
The Cellicon™ Perfusion Filter and Controller provide high throughput with low fouling and cell shear, and consistent process control ideal for the adoption of a N-1 perfused seed train.

The Cellvento® 4CHO-X Expansion Medium is specifically formulated to support seed train applications through N-1 including N-1 perfusion. It allows for optimal preparation of cells for production phases while supporting high cell growth at low cell-specific perfusion rates (CSPR) to increase productivity at the N-stage. When supplemented with DMSO, the Cellvento® 4CHO-X expansion medium acts as an ideal cryopreservation medium for use in HCDC process while minimizing cell adaption when coming in and out of the frozen stage.

For more information, visit
SigmaAldrich.com/Seed-Train

Single-Use Assembly Design

The Mobius® HCDC assembly is produced in two sizes: 100 mL bag assembly or 250 mL bag assembly. Both assemblies consist of the items listed below.



Component	Description	HCDC -80 °C Assembly Design	HCDC -196 °C Assembly Design
1	Waste Bag	NovaSeptum® bag 1000 mL (6 in. W x 12.3 in. H) End-ported (2 x ⅛ in.)	NovaSeptum® bag 1000 mL (6 in. W x 12.3 in. H) End-ported (2 x ⅛ in.)
2	Sample Bag	NovaSeptum® bag 100 mL (3.27 in. W x 6 in. H) End-ported (2 x ⅛ in.) or NovaSeptum® bag 250 mL (5.12 in. W x 6 in. H) End-ported (2 x ⅛ in.)	Fluoropolymer bag 100 mL (6.3 in. W x 3.9 in. H) End-ported (2 x ⅛ in.) or Flouropolymer bag 250 mL (9.4 in. W x 3.9 in. H) End-ported (2 x ⅛ in.)
3	Tubing	C-Flex® 374 (⅛ in. ID x ¼ in. OD)	C-Flex® 374 (⅛ in. ID x ¼ in. OD)
4	Tubing	C-Flex® 374 (⅛ in. ID x ¼ in. OD)	C-Flex® 374 (⅛ in. ID x ¼ in. OD)
5	Tubing	C-Flex® 374 (⅛ in. ID x ¼ in. OD)	C-Flex® 374 (⅛ in. ID x ¼ in. OD)
6	Tubing	C-Flex® 374 (⅛ in. ID x ¼ in. OD)	C-Flex® 374 (⅛ in. ID x ¼ in. OD)
7	Tubing	Pharma 65 (3 mm ID x 6 mm OD)	C-Flex® 374 (⅛ in. ID x ¼ in. OD)
8	Tubing	Pharma 65 (3 mm ID x 6 mm OD)	Pharma 65 (3 mm ID x 6 mm OD)
9	Tubing	Pharma 65 (3 mm ID x 6 mm OD)	N/A
10	Tubing	Pharma APT (3⁄16 in. ID x 5⁄16 in. OD)	N/A
11	Coupler	Straight reducer ¼ in. x ⅛ in.	N/A
12	Coupler	Tee ⅛ in.	Tee ⅛ in.
13	Plug-end	Plug-end ⅛ in.	Plug-end ⅛ in.
14	Clamp	Pinch Clamp, ½ in. (6 mm–⅛ in. OD to ⅜ in. OD tube)	Pinch Clamp, ½ in. (6 mm–⅛ in. OD to ⅜ in. OD tube)
15	Pinch Pipe	NovaSeal pinch pipe for 3 mm ID x 6 mm OD tubing	NovaSeal pinch pipe for 3 mm ID x 6 mm OD tubing

Specification

Design Criteria	
Component Material Toxicity	Family component materials were tested post gamma irradiation and meet the criteria for the USP <88>, Biological Reactivity Test for class VI plastics. These products also meet USP <661> for Physico-chemical tests for plastic containers.
Operating Temperature	HCDC -80 °C: -112–140 °F (-80–60 °C)* HCDC -196 °C: -321–140 °F (-196–60 °C)*
Release Criteria	
Critical Dimensions	Measurements are taken on assemblies to assure dimensional compliance with specifications and tolerances.
Quality Certification	Gold
Integrity Testing	Each assembly is leak integrity tested using a pressure decay method.
Appearance and Cleanliness	Each assembly is visually inspected with an unaided eye per manufacturing work instructions and test specifications. The assembly meets the Quality Assurance release criteria.
Sterility	Each assembly is sterilized using a validated gamma irradiation level of 25–40 kGy.
Bacterial Endotoxin	An assembly aqueous extraction contains less than 0.25 EU/mL per USP<85> as determined by the Limulus Amebeocyte Lysate (LAL) test.
Particulates	An assembly aqueous extraction has been tested and passed per USP<788>.

* When freezing the bag assembly, recommend bag and tubing be supported in a protective case for the entirety of the freezing and thawing process. Handle the cryobag with care at all times.

Fluid Path Materials of Construction

Component	Material of Construction
HCDC -80°C sample bag	PureFlex™ film, boat port, polyethylene
HCDC -196°C sample bag	Fluoropolymer film, boat port
C-Flex® 374 tubing	Thermoplastic elastomer
Silicone tubing	Platinum cured silicone
Coupler tee	Polypropylene
Plug end	Polypropylene

Ordering Information

Cat. No.	Product Description	Package Size
HCDC0001L101	Mobius® HCDC -80 °C assembly 100 mL	5 assemblies per package
HCDC0001L102	Mobius® HCDC -80 °C assembly 250 mL	5 assemblies per package
HCDC0001L0100	Mobius® HCDC -196 °C assembly 100 mL	5 assemblies per package
HCDC0001L0250	Mobius® HCDC -196 °C assembly 250 mL	5 assemblies per package

Supporting Materials

Cat. No.	Product Description	Package Size
NSF-10100	Protective Case for HCDC -80 °C 100 mL bag	25 per package
NSF-10250	Protective Case for HCDC -80 °C 250 mL bag	25 per package
KSET_1003[1]	Protective Case for HCDC -196 °C, 100 mL bag Protective Case for HCDC -196 °C, 250 mL bag	
KSET_1103[1]	Handling Rack for 5 x KSET_1003	

1. Product recommendation purchased separately from Single Use Support GmbH (**http://www.susupport.com/**).
Please contact your Merck representative for additional options.

BioContinuum™ Platform

Converge, Intensify and Evolve. Together.

An expanding environment of advanced processing, software, automation and analytic technologies, unlocking the potential of Bioprocessing 4.0 and empowering biomanufacturers to achieve greater speed, flexibility and quality.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

For additional information, visit **SigmaAldrich.com/Seed-Train**
To place an order or receive technical assistance, visit **SigmaAldrich.com/offices**

SigmaAldrich.com

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®

MK_DS7572EN Ver. 2.0
52668
01/2024