XCell ATF® 1 Device

Set-up Guide

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Abbreviations

A-B	Allen-Bradley
AC	Alternating current
Amp	Ampere
ATF	Alternating tangential flow
ATF-A	XCell ATF® Device A
ATF-B	XCell ATF® Device B
AUX	Auxiliary
A2B	XCell ATF® Device to Bioreactor connection
A2C	XCell ATF® Device to Controller connection
CFM	Cubic feet per meter
CSPR	Cell specific perfusion rate
dB	Decibels
DC	Direct current
DO	Dissolved oxygen
DPv1	PROFIBUS Decentralized Peripherals version 1
FAS	Field Applications Scientist
FC	Flow control
FIT	Filter integrity testing
FS	Flow sensor
FSE	Field Service Engineer
HFM	Hollow Fiber Module
HMI	Human Machine Interface
HP	Headplate
Hz	Hertz
ID	Inner diameter
I/O	Input/output
kg	Kilograms
L	Liter
lbs.	Pounds
LPM	Liters per minute
mA	Milliamp
mL	Milliliter
mV	Millivolt
NPT	National pipe thread
OD	Outer diameter
PCV	Pressure control valve
PID	Proportional, integral, and derivative
PLC	Programmable logic controller
POI	Product of interest
PRV	Pressure regulating valve
PV	Process value
P2	PCV outlet pressure (Commanded pressure)
P3	Permeate pressure
PPE	Personal protective equipment
psi	Pounds per square inch

psig	Pounds per square inch gauge
QC	Quick Connect
SAPA	Supply Air Protection Assembly
SCADA	Supervisory Control and Data Acquisition
SOP	Standard operating procedure
SP	Set point
SUB	Single-use Bioreactor
TC	Tri-clamp
TCD	Total cell density
TCP/IP	Transmission control protocol / Internet protocol
VCD	Viable cell density
VVD	Vessel volume exchange per day

Definitions

ATF rate	The rate at which cell culture is exchanged between the bioreactor and ATF Device. *ATF Rate (L/min) = Pump displacement volume (L) ÷ Cycle time (min)*
Filtration rate	The rate at which cell culture fluid flows across the hollow fiber membrane. The surface area of the hollow fiber membrane largely determines the value.

1. Introduction

XCell ATF® Technology provides a complete solution for cell retention within a bioreactor during cell culture processes. XCell ATF® Technology represents the gold standard for cell retention due to its effectiveness and proven reliability and scalability in commercial processes. Typical applications include:

- N-1 and High Productivity Harvest (HPH) intensification for fed-batch processes
- Vaccine and viral process intensification
- Perfusion for long-term continuous processing
- Gene therapy process intensification and media exchange

XCell ATF® Technology combines hardware, software, a filtration device, and an innovative pumping method to achieve the filtration result. Please direct questions regarding specific applications of the technology to your account manager, Field Application Scientist (FAS), or local authorized representative.

Small-scale bioreactors are often used in early R&D and process development to perform a wide range of studies, such as cell line characterization, media optimization, development of fed-batch intensification and perfusion applications as well as other intensified processes. The XCell ATF® 1 Device is designed to enable reliable scaled-down cell culture applications with linear flux and shear parameters for scale-up to 5000 L. The 0.5 - 2 L working volume may be leveraged to either reduce media cost or maintain cost with expansion and acceleration of the Design of Experiments (DoE). In conjunction with the XCell™ Lab Controller, the XCell ATF® 1 Device and its integrated accessories, including an AseptiQuik® S connector and a permeate pressure sensor, provide critical characterization data for optimizing processes during early development and as part of ongoing commercial scale operations.

All Hollow fiber membranes are made of polyethersulfone (PES) with 0.2 um pore size and 1 mm inner diameter. The shared properties (materials of construction, pore size, ID) ensure linear scalability across all device sizes.

XCell ATF® 1 Devices are provided dry and gamma irradiated/pre-sterilized. Complete wetting is recommended (after installation but prior to use) to ensure optimal hollow fiber filter (HFF) performance.

This document describes the set-up and connectivity of the XCell ATF® 1 Single-use Device, serving as a reference for making appropriate connections, preparing the device, and initiating the cell culture process with sterility throughout the fluid path. This guide assumes that users possess a basic level of skill and knowledge in the areas of aseptic technique, fluid handling and use of XCell ATF® Devices.

This guide is not intended to provide optimization guidance for the XCell ATF® 1 Device operation or the cell culture process. Section 8 describes proper set-up and operation of the XCell ATF® 1 Device. For further support in optimizing or troubleshooting, please contact your local Repligen Field Applications Scientist (FAS).

2. About this document

This manual uses several different phrases. Each phrase should draw the following level of attention.

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or instrument damage if the information is not heeded.
WARNING!	Warns users of potential serious physical injury if warnings are not heeded.

3. Safety precautions

Table 2. Explanation of symbols

Phrase	Symbol	Description
IMPORTANT:		The XCell ATF® 1 Device is designed to be single use. Any attempt to misuse, reuse, or disassemble it will likely result in a loss of sterility and integrity causing leakage, culture contamination, poor performance and/or damage to the XCell ATF® 1 Device.
IMPORTANT:		**Sterility:** The connection between XCell ATF® 1 Device and bioreactor is made utilizing a CPC AseptiQuik® S sterile connector. It is important to use sterile techniques when connecting and securing the aseptic connector assembly. For wetting and installation, multiple sterile flow path configurations may need to be established. Isolate and clamp tubes along the flow path to maintain sterility.
IMPORTANT:		Ensure the built-in clamps are correctly positioned to establish the requisite flow paths. For best performance, inspect the tubing carefully each time the clamps are removed to ensure the tubing are not crimped. If necessary, gently roll the tubing to re-establish proper flow.
IMPORTANT:		The total length of the A2B tubing from the top of the XCell ATF® 1 Device to the bioreactor headplate must not exceed 14”.
WARNING:		**Power:** Use only Repligen the provided power supply. • Use only high voltage cord specific for your region provided by Repligen. • Do not use a damaged power supply. Do not use a damaged power cord.
WARNING:		**Tubing:** Tubing breakage between the XCell ATF® 1 Device and bioreactor may result in fluid spraying from the pump. Use appropriate measures to protect the operator and equipment.
WARNING:		Wear standard laboratory PPE, including lab coat, protective eye wear and gloves.

Note: *Please refer to the XCell™ Lab Controller User Guide for controller-related precautions.*

4. Product description and accessories

The XCell ATF® 1 Device includes a hollow fiber filter seated above a diaphragm pump with integrated tube sets and accessories, such as a CPC AseptiQuik® S connector and permeate pressure sensor (Figure 1). A supporting stainless-steel stand ensures operational stability of the XCell ATF® 1 Device.

4.1 Single-use Device

Figure 1. XCell ATF® 1 Device components

1. CPC AseptiQuik® S Connector
2. ATF to Bioreactor (A2B)
3. Permeate pressure sensor (SciLog® SciPres®)
4. Top permeate
5. XCell ATF® filter housing
6. Bottom permeate
7. Diaphragm
8. ATF to Controller (A2C)

Diaphragm pump
The diaphragm pump consists of a spherical chamber at the base of the XCell ATF® 1 Device. A silicone diaphragm separates the air side hemisphere and the liquid side hemisphere of the pump. The silicone diaphragm moves up and down as either pressurized air or vacuum is applied to the air side of the pump. As the diaphragm pump cycles through exhaust and intake, cell culture suspension moves from the bioreactor to the XCell ATF® 1 Device and back to the bioreactor. This alternating tangential flow draws cell suspension in a continuous back and forth motion through the lumen of the hollow fiber filters. Alternating flow creates a beneficial backflush, enabling filter self-cleaning that minimizes fouling.

Filter module
The filter module encloses a hollow fiber filter within a housing that plumbs liquid. The hollow fiber filter, sitting above the liquid side hemisphere of the diaphragm pump, separates media from cells. The filter housing contains four ports: two permeate ports (top and bottom), connections from the ATF to Controller (A2C) and ATF to Bioreactor (A2B).

Note: *The top permeate port must be used to pull permeate, while the bottom port can be clamped off.*

Stainless-steel clamp-on stand
To ensure XCell ATF® 1 Device stability during setup and operation, the use of the purpose-built stainless-steel stand (see Figure 2) is recommended by Repligen. The stand secures the device and enables proper positioning of the A2B tubing.

Figure 2. Support stand for XCell ATF® 1 Device

1. A2B Flow Sensor
2. Stainless-steel stand
3. 0.2 µm PES filter
4. Benchtop attachment mount
5. ABS clamp for filter housing

The stand consists of a stainless-steel rod with multiple clamps. Two ABS clamps hold the XCell ATF® 1 Device vertically, one stainless-steel sheet metal clamp holds the flow sensor, and a final clamp secures the stand itself to the bench.

4.2 Connection kit (tubing sets)

XCell ATF® 1 Device is manufactured with the tubing elements required for connectivity to both the bioreactor and the XCell™ Lab Controller. The tubing elements are equipped with pinch clamps that can be used to create different fluid paths.

A2B tubing and flow sensor
The A2B port connects the XCell ATF® 1 Device to the Bioreactor using the A2B tubing. The A2B tubing consists of 9” of C-Flex® 374 tubing terminated with a CPC AseptiQuik® S 1/8” I.D. connector. If the bioreactor is also equipped with a CPC AseptiQuik® S 1/8” I.D. connector, a sterile connection may be made following the manufacturer’s instructions. Alternatively, the A2B tubing can be welded into the flow path of the bioreactor, using the appropriate welder.

Note: *The total length of the A2B connection should be limited to 14” (~36 cm) for optimal performance.*

A flow sensor (optional) integrated into the A2B line, measures feed/retentate flow and provides real-time Device performance monitoring.

A2C tubing
The A2C port connects the XCell ATF® Device to the Controller using the A2C tubing. Positioned at the lowest point on the pump base, the A2C port delivers pressurized air and vacuum to the pump. No liquid passes through the A2C port.

Permeate tubing and pressure sensor
The XCell ATF® 1 Device includes two permeate ports. The top permeate port is typically used for pulling permeate fluid. A pressure sensor (optional) integrated into the top permeate port enables permeate pressure measurement for assessing real-time filter performance and filter exchange. The bottom permeate port can be aseptically welded to a vessel for post-use draining of the device. Permeate ports are supplied with a capped weldable 1/8" I.D. PureWeld® XL tubing to connect collection bags.

4.3 System specifications

Table 3. Part Numbers for the XCell ATF® 1 Single-use Device

Part number	Description
suATF1-S02PES	XCell ATF® 1 Single-use Device, 61cm, 0.2µ PES
suATF1-S02PES-P	XCell ATF® 1 Single-use Device, 61cm, 0.2µ PES w/ Pressure Sensor
suATF12-STAND-CO	XCell ATF® 1 and XCell ATF® 2 Single-use Device Clamp-On Stand
B-DIP-3-200	Barbed Diptube, 1/8" ID, 200 mm Length
HP-ATF1-6-M10	Head plate fitting, 1/8" ID, 6 mm port, M10
HP-ATF1-12-PG135	Head plate fitting, 1/8" ID, 12 mm port, PG13.5

4.4 Materials of construction

Table 4. Materials of construction for product contact parts

Non-Product Contact Parts	Materials of Construction
Air Filter Membrane and Cartridge	PVDF filter - Polyvinylidene difluoride, Polypropylene
A2C tube set	Polyurethane, Polypropylene, PVDF
Sanitary Clamps	Glass-Filled Nylon
Pinch Clamps	Polypropylene, Polyester
Tie Wraps	Nylon
Tubing Clamps	Stainless Steel
Stand	Acrylonitrile Butadiene Styrene (ABS), Stainless Steel
Adhesives	Cyanoacrylate, Acrylic

Table 5. Tubing materials of construction and specifications

Tubing type	Gamma irradiated	Tubing spec (ID, size or OD, length)	Materials of construction	Connection type	Accessories
A2B	yes	1/8" ID, size 16, 9" long	C-Flex® 374	CPC AseptiQuik® S 1/8" connector	Flow sensor (optional), pinch clamp
Top permeate	yes	1/8" ID, size 16, 48" long	PureWeld® XL	Terminal end cap	Pinch clamp, P3 pressure sensor (optional)
Bottom permeate	yes	1/8" ID, size 16, 18" long	PureWeld® XL	Terminal end cap	Pinch clamp
A2C	N/A	5/32" ID, ¼" OD, 7" long	Polyurethane	Push-to-connect fitting	0.2µ PVDF filter

5. Prerequisites for operation

The following equipment not supplied with the XCell ATF® 1 Device are required to operate the device.

For XCell ATF® 1 Device:

- An operational, calibrated XCell™ Lab Controller, connected to the required air pressure and vacuum sources.
- Dip tube and sterile connector if the bioreactor (typically glass) does not come with one. Tubing ID should be 1/8” and length ≤5”.

For permeate (harvest) collection:

- A variable speed peristaltic pump capable of supporting flow rates in the range 0.1 - 20 mL/min. The exact flow rate depends on the bioreactor working volume and perfusion rate.
- An appropriate length of 1/8” I.D. weldable tubing on a collection bag. It is recommended that the permeate line of the collection bag be sterilized via gamma irradiation or autoclaving prior to use.

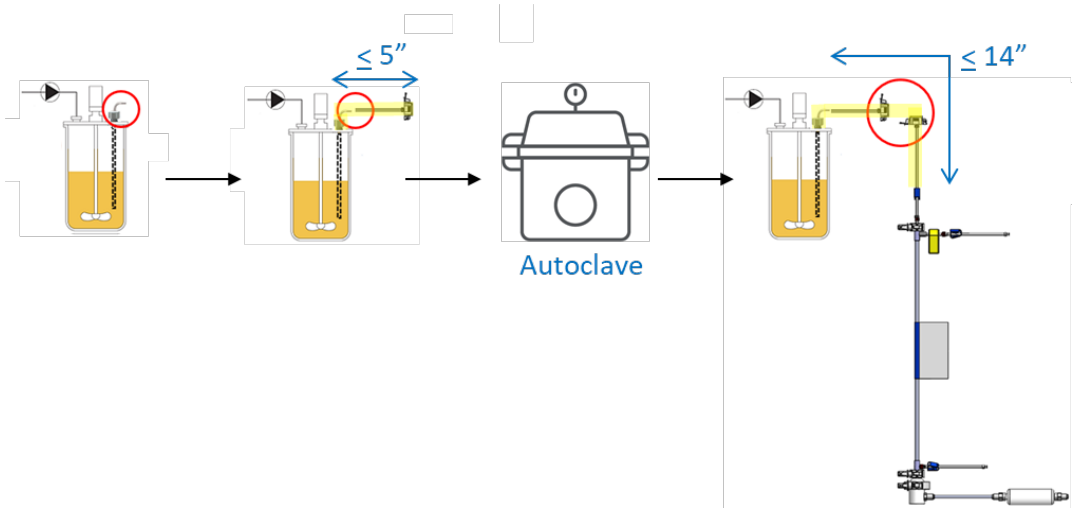
For filter wetting:

- An empty, sterile 1 L container or bag configured with size 16 (1/8” I.D.) weldable tubing to collect the wetting solution from the HFF permeate port.
- A variable speed peristaltic pump capable of supporting flow rates in the range 5 - 40 mL/min and accommodating tubing IDs configured on the 1 L container.

6. XCell ATF® 1 Device connection methods

6.1 Connection to a glass bioreactor

Figure 3. Connecting XCell ATF® 1 Device to a glass bioreactor



1. Insert the dip tube into the glass bioreactor using head plate fittings.
2. Attach tubing to the head plate fitting followed by a sterile connector (refer to Table 2).

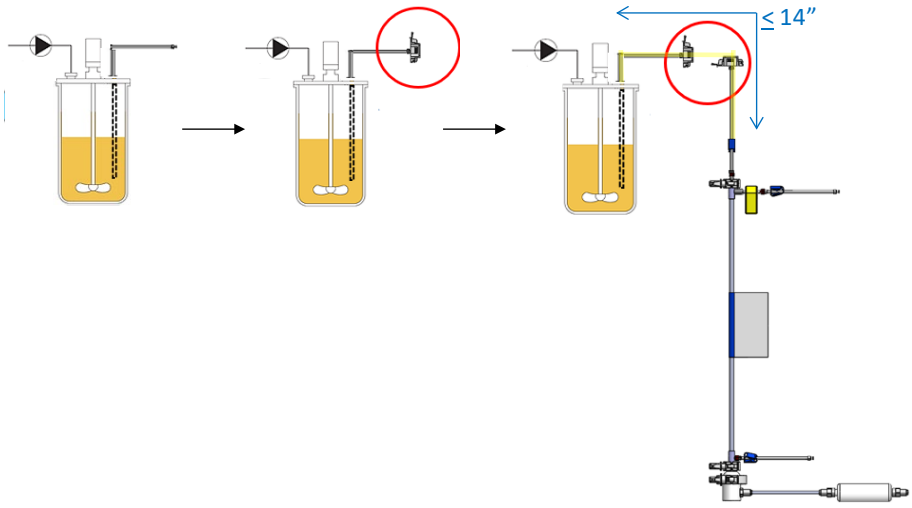
Note: *Tube set, and sterile connector are not supplied by Repligen. Tubing ID should be 1/8” and max length ≤5”.*

3. Autoclave the entire set-up including the bioreactor.
4. Connect the gamma irradiated XCell ATF® 1 Device to the bioreactor using the CPC AseptiQuik® S sterile connector.

Note: *The total length of the A2B tube from the top of the XCell ATF® Device to the bioreactor headplate must not exceed 14”.*

6.2 Connection to a single-use bioreactor

Figure 4. Connecting XCell ATF® 1 Device to a single-use bioreactor



Most single-use bench scale bioreactors are equipped with a dip tube and associated tubing. Figure 4 illustrates how to connect the XCell ATF® 1 Device to a single-use bioreactor.

1. Connect aseptic connector to the tubing.
2. Connect the XCell ATF® 1 Device to the bioreactor using the CPC AseptiQuik® S sterile connector.

Note: *The total length of the A2B tube from the top of the XCell ATF® 1 Device to the bioreactor headplate must not exceed 14”. If unable to meet the requirements, please consult with your local Field Applications Specialist (FAS).*

7. Hollow fiber filter wetting

The XCell ATF® 1 Device is supplied dry. During filter integrity testing (FIT) the filter is pre-wet, drained and integrity tested. After FIT, the filter is dried to a fixed weight specification. Repligen recommends pre-wetting the hollow fiber filter (HFF) before use to ensure robust performance. Proper HFF wetting requires a minimum of 500 mL of sterile culture media.

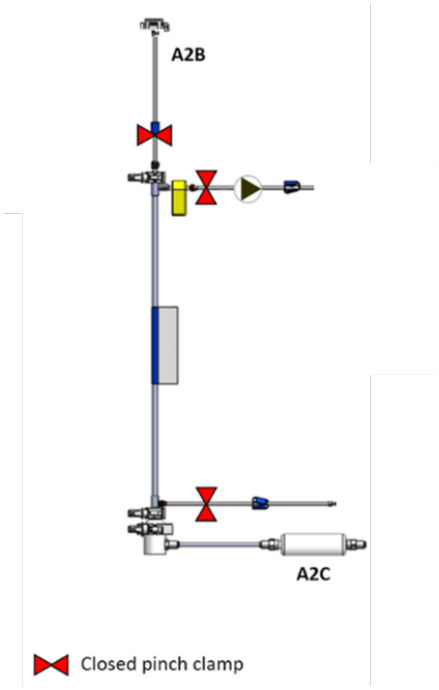
7.1 Wetting procedure

The XCell ATF® 1 Device wetting procedure is executed with the device connected to a bioreactor that contains cell culture media (pre-inoculation). The following method utilizes the XCell™ Lab Controller to actuate the pump for filter wetting.

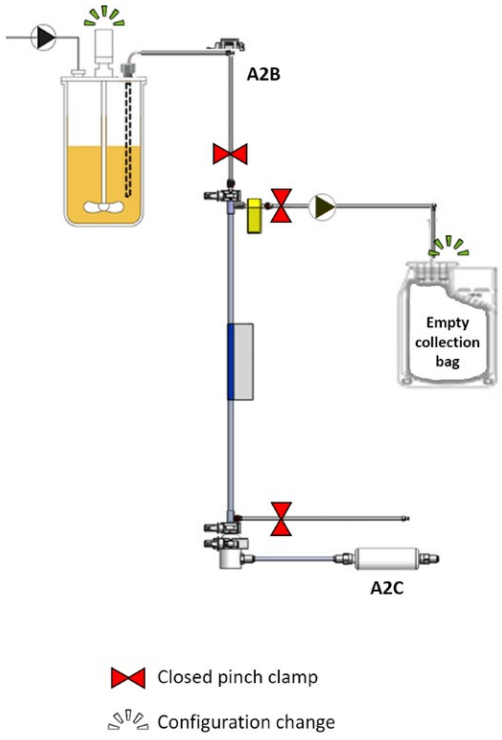
1. Prepare a sterile bioreactor according to your standard operating procedure (SOP). The bioreactor must contain a minimum of 1000 mL sterile cell culture media and be cell-free (pre-inoculation).

Note: *The device wetting must be executed prior to bioreactor inoculation.*

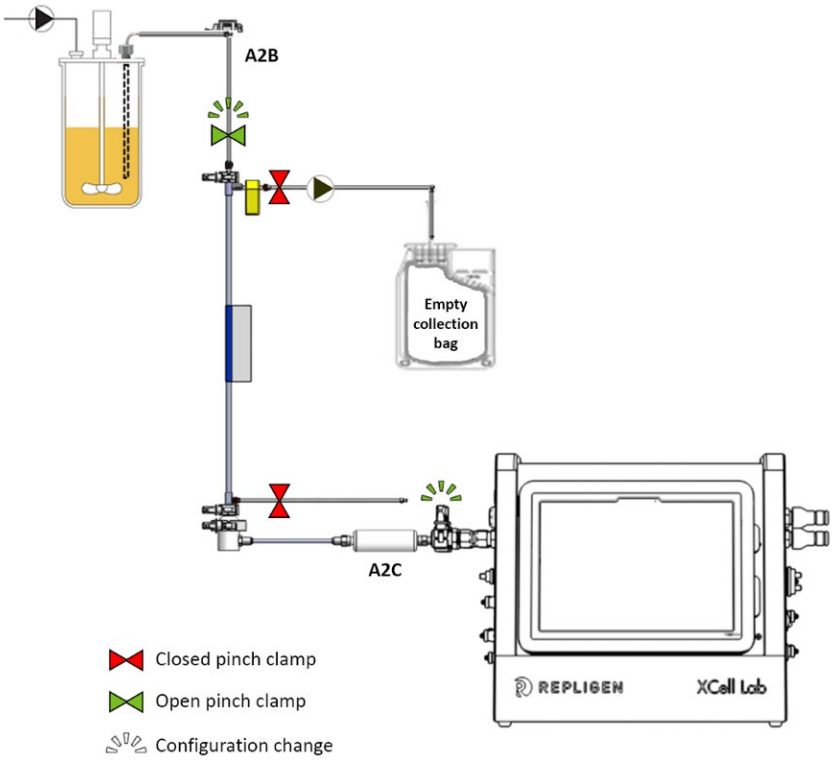
2. Secure the XCell ATF® 1 Device in the stand. Ensure pinch clamps on all tubing sets, including those on the A2B, are clamped (see figure).



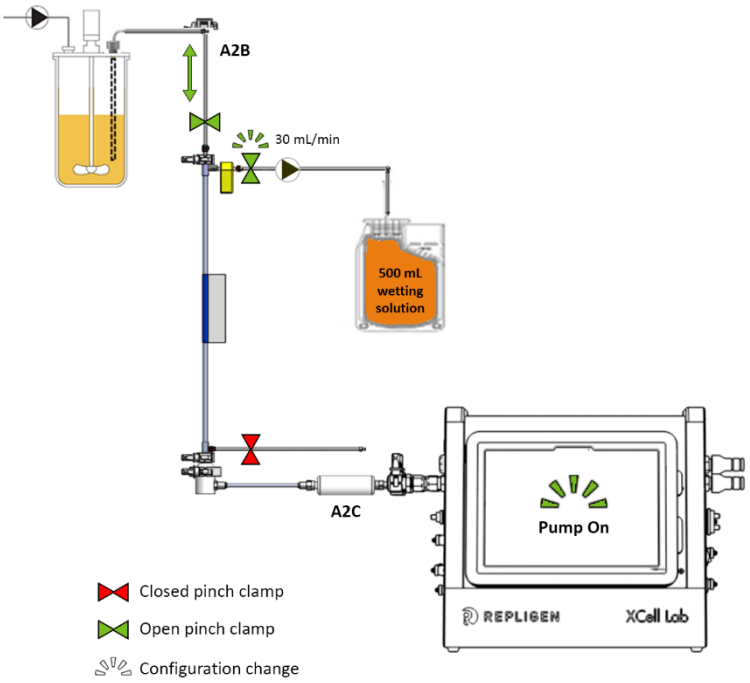
3. Connect the XCell ATF® 1 Device to the bioreactor using a sterile connection (See Section 8).
4. Prepare an empty 1 L sterile container fitted with an appropriate length of 1/8” I.D. weldable tubing.
5. Weld the tubing of the 1 L sterile container to the top permeate port of the XCell ATF® 1 Device (see figure).



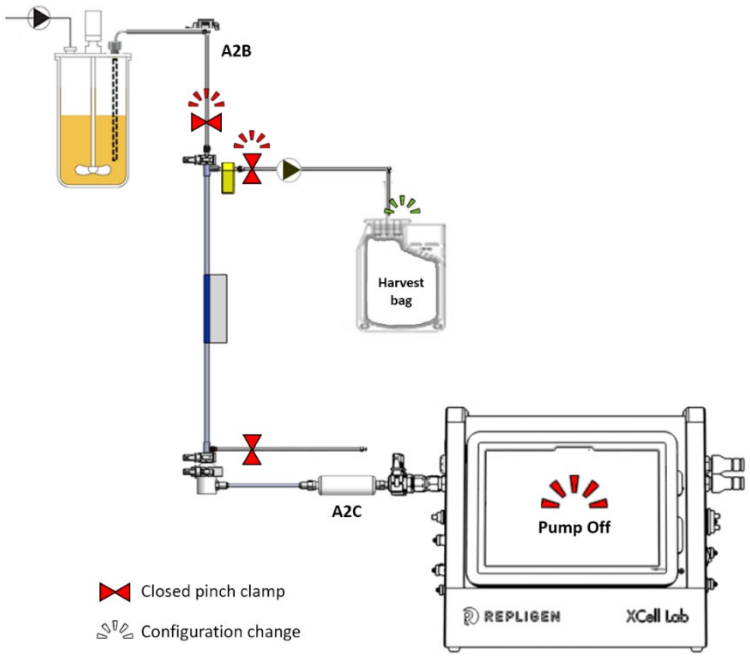
6. Open the flow path between the bioreactor and device by releasing the A2B tubing segment clamp.
7. Connect the XCell™ Lab Controller to the device using the A2C tubing. Refer to the XCell™ Lab Controller User Guide for additional instructions on controller operation.



8. Set the ATF flow rate on the XCell™ Lab Controller software to 0.14 LPM and let the pump run for a few minutes until the flow set point is reached.
9. After the target flow rate is achieved, release the pinch clamp on the top permeate port and immediately start running the permeate pump at a flow rate of 30 mL/min.
10. Collect at least 500 mL of wetting solution in the permeate collection container.



11. After completing the wetting process, stop the permeate pump and then the ATF pump. Clamp the A2B tubing line and replace the collection container with a new empty bag using tube welding.

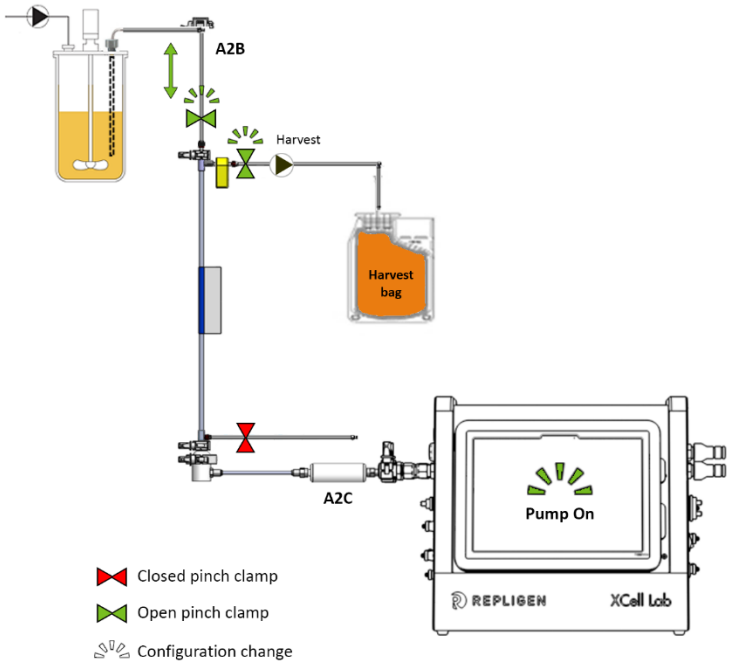


12. Media in the wetting bag can be incubated overnight to determine sterility of the XCell ATF® 1 Device.
13. The remaining media filled Device can be left until the bioreactor inoculation.

8. Operational configuration

Figure 5 illustrates the XCell ATF® 1 Device configuration during harvest. It is important to note the tubing clamp configuration for proper operation and sterility maintenance.

Figure 5. Configuration of an XCell ATF ® 1 Device during harvesting



8.1 XCell ATF® 1 Device operation

Follow these steps to properly set-up and operate the XCell ATF® 1 Device:

Support stand

- Adjust the rod height before attaching the XCell ATF® 1 Device. Use the smaller screw at the back of the clamp to achieve the desired height (Figure 2).
- Ensure the filter is secured at positions just above the bottom endcap and just below the top endcap. Use the ABS clamps and thumb screws to hold the XCell ATF® 1 Device securely in place.
- Ensure the support stand is fixed adequately to the bench. We recommend using appropriate mount provided with the XCell ATF® 1 Device.

A2B and permeate tubing set

- Ensure any tubing to be welded onto the device is 1/8" I.D. and size 16 weldable tubing.
- Ensure the A2B tubing is fitted with a CPC AseptiQuik® S 1/8" sterile connector.
- Ensure the flow sensor is secured in the stainless-steel support stand.
- Ensure electrical cables carrying data from the flow sensor(s) and/or the pressure sensor(s) are connected properly to the sensor and controller.
- Ensure the A2B tubing is not pinched or crimped along the flow path.
- The total length of tubing from the top of the XCell ATF® 1 Device to the bioreactor (excluding the dip tube) must not exceed 14". The A2B tubing on the device is 9" long. The tubing length from the bioreactor must be ≤ 5".
- Ensure arrows on the flow sensor point away from the XCell ATF® 1 Device.

A2C

- Ensure A2C air tubing is fully inserted into the push-to-connect fitting on the A2C air filter.

Flow rates

- ATF rates of 0.10 - 0.14 L/min are a good starting point for most processes. Shear-sensitive cell lines may respond better to lower ATF rates between 0.07 - 0.10 L/min.
- Typical perfusion rates range from 0.5 - 3.0 vessel volumes per day (VVD) with most users starting perfusion 2-3 days after inoculation at 0.5 - 1.0 VVD. The N-1 application uses similar perfusion rate with a much shorter duration, typically 3 - 7 days.
- The High Productivity Harvest application typically uses harvest rates of 0.1 - 2.0 VVD for 24-96 hours.
- Media exchange process recommendations include permeate rates of 0.25 - 1.0 VVH.
- To keep the bioreactor volume constant, the incoming media/feed rate must equal the harvest rate. Constant bioreactor volume can be achieved using weight-based controls, level probes, or automated pump control to synchronize harvest and feed pumping rates.
- Critical variables affecting flow and filtration rates include culture volume, cell density, viability, cell line properties, process duration and shear sensitivity.
- For process scale-up support, please contact your local Field Applications Scientist (FAS). A complete XCell ATF® Engineering Scale-up Guide is available upon request.
- It is important to note that higher harvest rates require higher ATF flow rates. This relationship is represented by the following equation:

$$\text{Ratio} = \text{ATF Flow rate (L/min)} / \text{Filtration rate (L/min)}$$

  Higher ratios are preferred to help reduce filter surface film layer buildup.

- For first-time users, Repligen recommends setting up the run using default ATF flow rates provided in the XCell™ Lab Controller User Guide.

9. Post-use instructions

Execute the following steps to discard the XCell ATF® 1 Device after completion of a run:

1. Stop the permeate pump and clamp the tubing line.
2. Disconnect the collection bag in a sterile manner.
3. Stop ATF flow and disconnect the XCell ATF® 1 Device from the A2C line using the push-to-connect fitting on the air filter (side facing XCell™ Lab Controller).
4. Clamp the A2B line using a pinch clamp.
5. Disconnect the XCell ATF® 1 Device from the bioreactor and discard the cell culture solution by following proper waste disposal procedures.

10. Frequently asked questions

What if a leak is detected during the wetting procedure?
Each individual XCell ATF® 1 Device is pressure-tested at 25 psi to ensure the integrity of the entire assembly. However, if a leak is detected during the wetting procedure, stop the ATF flow immediately and attempt to identify the location of the leak. Ensure that pinch clamps are properly installed at appropriate locations. Clamping at the wrong locations during the wetting procedure can pressurize the Device and cause leakage. If no errors or inaccuracies can be found in the setup, contact a local Repligen Sales Manager or Repligen Customer Support for further assistance.

How do I ensure the sterility of a XCell ATF® 1 Single-use Device?
The wetting solution collected from the filter wetting procedure can be incubated in a shake flask at 37° C for 24 hours to assess the sterility of the XCell ATF® 1 Device.

How long can the XCell ATF® 1 Single-use Device be stored wet before use in the perfusion process?
After completing the wetting procedure, all tubing segments—including the A2B—must be closed using pinch clamps. The XCell ATF® 1 Device can be stored wet for about one week before initiating the perfusion process. It is important that sterility is maintained throughout set-up and storage.

11. Index