

# XCell® ATF 6 Single-use Devices

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## User Guide

For use with XCell® LS Controllers or C410 Controllers



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### Abbreviations

ATF	Alternating Tangential Flow
A2B	XCell ATF Device to Bioreactor connection
A2C	XCell ATF Device to Controller connection
DDVR	Displacement to dead volume ratio
FAS	Field Applications Scientist
FIT	Filter integrity testing
ID	Inner diameter
L	Liter
LPM	Liters per minute
LSC	Large Scale Controller
mL	Milliliter
PPE	Personal protective equipment
psi	Pounds per square inch
SP	Set point
SUB	Single-use Bioreactor
TC	Tri-clamp

### Definitions

**ATF Rate (ATF Diaphragm Pump Rate):** Rate at which cell culture is exchanged between the bioreactor and XCell ATF Device.

*ATF Rate (L/min) = Pump displacement volume (L) / Cycle time (min).*

**Filtration Rate (Permeate Flow Rate):** Rate at which cell free fluid flows across the hollow fiber membrane. The surface area of the hollow fiber membrane largely determines the value.

## 1. Introduction

XCell® ATF Technology uses alternating tangential flow (ATF) to intensify upstream processes by retaining cells in the bioreactor while continuously perfusing fresh media and removing spent media with the biological target. An innovative diaphragm pump creates alternating tangential flow across a hollow fiber filter cell retention device, leading to high viable cell densities and increased productivity with lower cost of goods. XCell ATF Technology can be used to intensify Cell Banking, N-1, N-stage and continuous cell culture processes. The technology has been applied to many cell lines and is used for the production of recombinant proteins, monoclonal antibodies, vaccines, viral vectors, cell therapies, and cultivated food.

XCell ATF Technology enables production of high density, high viability cell cultures from 0.5 L to 5000 L working volumes. The ATF diaphragm pump delivers low shear, ensuring the attainment of high viable cell density. The system includes XCell ATF Devices in both single-use and stainless-steel formats, XCell LS Controllers, tubing set kits for optimal connectivity, flow sensors, pressure sensors, and other accessories.

Hollow fiber filters (HFF) used in XCell ATF Devices are made of polyethersulfone (PES) with a 0.2 µm pore size and 1 mm lumen inner diameter (ID). These combined properties (materials of construction, pore size, ID) ensure cell retention, low shear, and effective sieving of the biologic of interest. HFF formats are scalable with increasing surface area/size and maintenance of the filter effective length, ensuring robust performance across ATF sizes and cell culture volumes.

Single-use tubing set kits and accessories are available for the connection of XCell ATF 6 Single-use Devices to both stainless steel and single-use bioreactors, simplifying preparation and connectivity and allowing for the proper exchange of cell culture material between the XCell ATF Device and the bioreactor and for harvesting product during production operations. The ATF controllers operate the diaphragm pump at the base of the device. The devices and tubing set kits are also compatible with both legacy C410 and XCell LS Controllers. Additional information on the use of the controllers and XCell ATF Technology can be found XCell LS Controller and C410 User Guide.

This document describes the major components, setup, and connectivity of the XCell ATF 6 Single-use Devices, 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 and fluid handling.

For further support in optimizing or troubleshooting, please contact your local Repligen Field Applications Specialist (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
<b>Note:</b>	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:		<b>The XCell ATF 6 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 6 Device. The device should not be autoclaved.</b>
IMPORTANT:		Sterility: The connection between XCell ATF 6 Device and bioreactor is made using CPC AseptiQuik® sterile connectors. 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 with external tubing clamps to maintain sterility.
IMPORTANT:		Ensure the 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 is not crimped. If necessary, gently roll the tubing to re-establish proper flow.
IMPORTANT:		<b>The total length of the A2B tubing from the top of the XCell ATF Single-Use Device to the bioreactor port must not exceed 40" for both XCell ATF 6 Single-Use Devices.</b>
WARNING:		Power: Use only Repligen the provided power supply. <ul style="list-style-type: none"> <li>• Use only power cord specific for your region provided by Repligen</li> <li>• Do not use a damaged power supply</li> <li>• Do not use a damaged power cord</li> </ul>
WARNING:		Tubing: Tubing breakage or a faulty connector between the XCell ATF 6 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, such as lab coat, protective eye wear, and gloves, as required by your facility.

**Note:** Please refer to the XCell LS and C410 Controller User Guides for controller-related precautions.

## 4. XCell ATF Devices

XCell ATF Large-scale Molded Devices include a hollow fiber filter seated above a diaphragm pump, tubing set kits to ensure sterile connectivity, and accessories such flow sensors and permeate pressure sensors. A stainless-steel stand is required for use to ensure the operational stability of the single-use XCell ATF 6 Devices. XCell ATF Devices are sold separately.

XCell ATF Large-scale Devices are supplied pre-sterilized and require wetting with sterile cell culture media or WFI prior to use.

**Note:** The devices are designed to be single-use and are therefore not designed to be cleaned, sanitized, or stored for repeat use. The device should not be exposed to high pH solutions, such as sodium hydroxide for extended time. Do not expose the device to even dilute caustic solutions prior to use.

### 4.1 XCell ATF Devices Pump Cycle

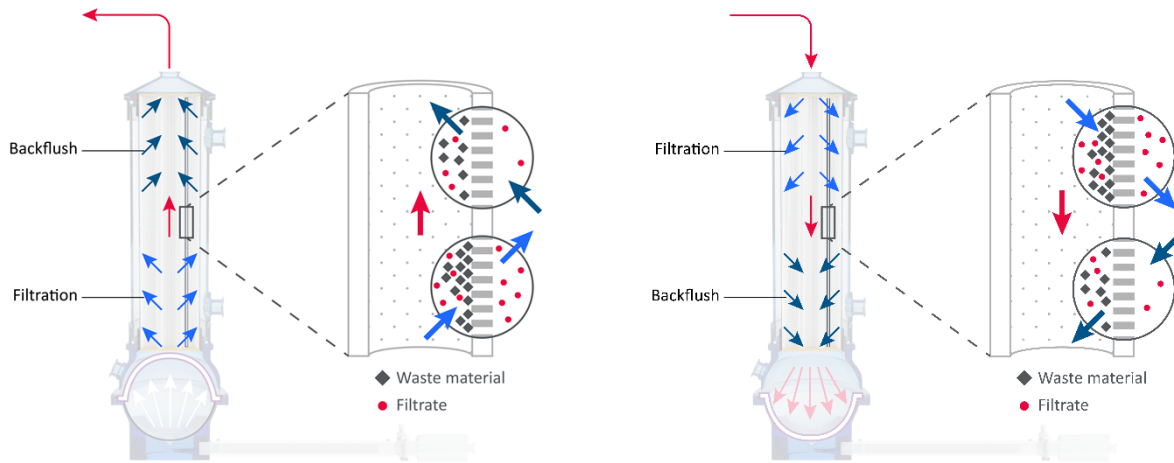
The diaphragm pump generates alternating tangential flow (ATF) through the hollow fiber filter. ATF is a pulsating, reversible flow of liquid between a bioreactor and diaphragm pump.

The process consists of two cycles ([Figure 1](#)), the pressure cycle (P-cycle) and the exhaust cycle (E-cycle). The P-cycle occurs when air is fed into the ATF pump, moving the diaphragm up towards the pump liquid-side (PL) hemisphere and driving the liquid from the diaphragm pump through the hollow fiber filter and into the bioreactor.

The E-cycle occurs when vacuum is introduced, thus pulling the diaphragm down towards the pump air-side (PA) hemisphere, and liquid is pulled from the bioreactor through the HFF, and back into the diaphragm pump. The diaphragm must travel between the

pressure and exhaust strokes to complete one cycle. The flow through the hollow fiber filter generates tangential flow in each direction in an alternating fashion.

**Figure 1. Pressure Strokes (Left) and Exhaust Strokes (Right)**



**Note:** XCell ATF Devices require a vacuum (negative pressure) to move the diaphragm to its lowest position. Positive pressure (overlay plus liquid head pressure) from the bioreactor is insufficient to completely deflate the diaphragm, necessitating the use of vacuum to ensure proper XCell ATF Device operation.

## 5. XCell ATF 6 Single-use Device

The XCell ATF 6 Single-use Device is recommended for process volumes of 50 – 200 L. Components include a diaphragm pump, filter housing, inlet elbow, and a hollow fiber filter cartridge fitted within the filter housing. The XCell ATF 6 PES Device is available with AseptiQuik aseptic connectors ([Table 3](#)).

**Table 3. XCell ATF 6 Ordering Information**

Part Number	Description	Notes
SUATF6-R02P-A	XCell ATF 6 Single-use Device, 0.2 µm PES	0.2 µm filter, CPC AseptiQuik aseptic connections

### 5.1 XCell ATF 6 Single-use Device Configuration

The diaphragm pump ([Figure 2. XCell ATF 6 Single-use Device Components](#)) consists of a spherical chamber at the base of the 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 device and back to the bioreactor. This alternating tangential flow draws cell suspension from the bioreactor in a continuous back and forth motion through the A2B connection and through the lumen of the hollow fiber filters. Alternating flow creates a backflush, with liquid moving from the permeate to the retentate across the hollow fiber wall, enabling the self-cleaning of the filter, that minimizes fouling and improves long term sieving performance.

The filter module contains a hollow fiber filter within a housing. The hollow fiber filter, sitting above the liquid side hemisphere of the diaphragm pump, retains cell suspension and therefore separates media from cells. The filter housing contains four (4) ports: two permeate ports (top and bottom), a condition/drain port, and an A2B port to connect the XCell ATF Device to the bioreactor. The XCell LS Controller or C410 Controller is connected to the XCell ATF Device via the A2C connection, enabling the pneumatic connection to the ATF device.

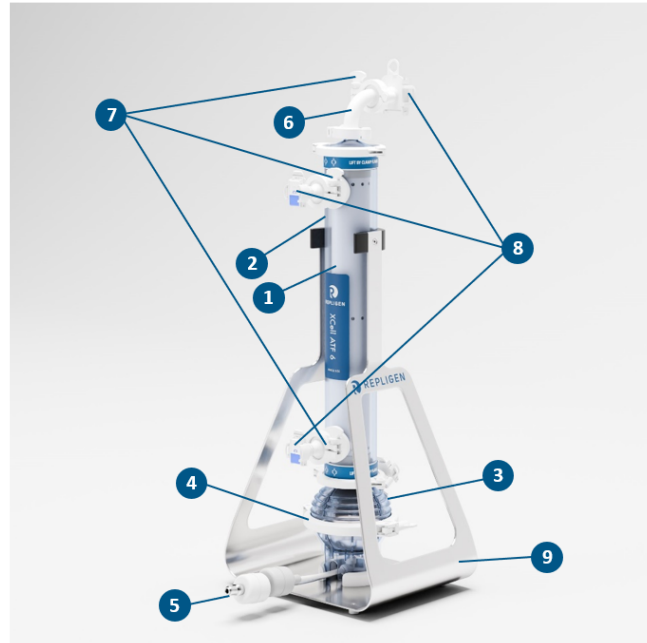
**Note:** The top permeate port directs permeate to the harvest vessel, while the bottom port may be clamped off.

To ensure stability during setup and use, it is recommended that the device be placed in the stainless-steel stand (Figure 2, Figure 3). The stand features a ring to hold the bottom of the device securely and a snap ring to hold the filter housing near the top of the device. The stand is provided with a notch to orient and secure the ATF to controller (A2C) line and to provide access to all ports.

**Note:** There are specific stainless-steel stands for the XCell ATF 6 Single-use devices and they must be ordered separately from the devices themselves. The stainless-steel stands are re-usable.

**Figure 2. XCell ATF 6 Single-use Device Components**

1. Hollow fiber filter
2. Housing
3. Diaphragm pump
4. Pump closure clamp
5. Hydrophobic vent filter; A2C connection
6. Inlet elbow; A2B connection
7. Sanitary clamps
8. Aseptic connectors, Permeate connections
9. Stainless steel stand



## 5.2 Required Equipment

The following equipment is required to operate the XCell ATF 6 Single-use Device:

- Control Hardware
  - XCell LS Controller or C140 Controller configured as required.
- Permeate (Harvest) Fluid Management
  - A variable-speed, peristaltic pump that supports flow rates of 50 – 400 mL/min, depending on the bioreactor working volume and perfusion rate.
  - A minimum length of ¼ inch ID tubing, fitted with a single aseptic connector to mate with the XCell ATF 6 Single-use Device. It is recommended that the permeate line be sterilized via gamma irradiation or autoclaving.
  - If the permeate will not be collected, it is still recommended to sterile filter the permeate to prevent contamination of the ATF device and ultimately the bioreactor. The permeate line should not be directed to a floor drain without a sterile filter in line.
- Pre-use, Offline Filter Wetting Equipment
  - A sterile 50 L empty single-use biocontainer with an appropriate length of tubing, a clamp, and a single, terminal aseptic connector.
  - Filtered WFI or cell culture media (50 L) dispensed into the 50 L single-use biocontainer.
  - A variable speed peristaltic pump that supports flow rates of 1 – 4 LPM and accommodates the tubing ID used with the media/WFI bag.



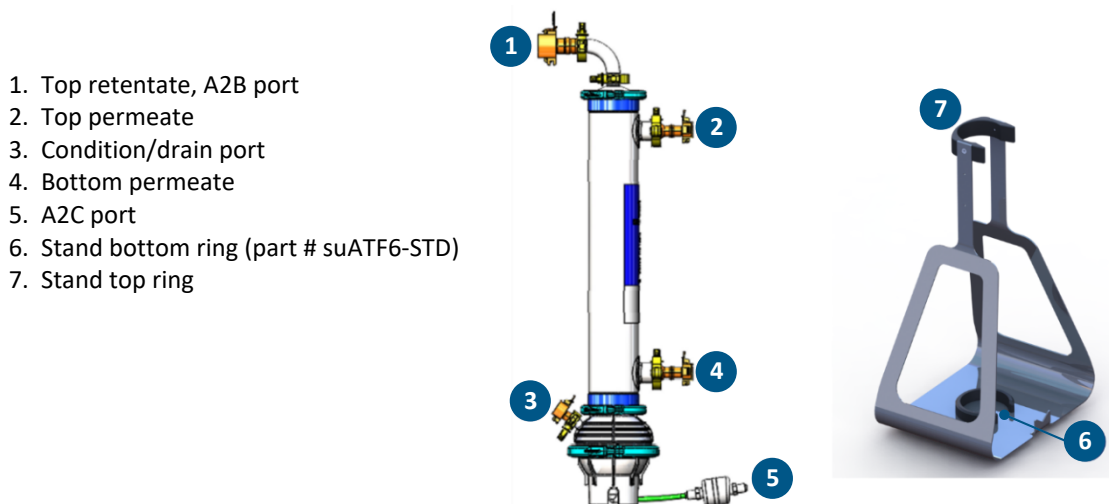
### 5.3 Device Ports

The device contains five ports/connection points (Figure 3):

- Top retentate (A2B) port
- Condition/drain port
- Top permeate port
- Bottom permeate port
- XCell ATF Device to XCell ATF LS Controller or C410 Controller (A2C) connection point

All ports, except the A2C port, are supplied dead-ended with AseptiQuik aseptic connectors. The A2C port, dead-ended with a vent filter and located at the lowest point on the pump base, is connected to the XCell LS Controller or C410 Controller. To establish a secure connection, insert the A2C line of the controller into the vent filter port via push-to-connect fitting. For optimal ATF performance, it is crucial to ensure that the A2C line is correctly positioned and fully inserted. The remaining four ports carry liquid between the XCell ATF Device and bioreactor or the XCell ATF Device and the permeate vessel. Two ports are connected to the feed, or retentate, side of the cartridge and two ports are connected to the permeate side. The top retentate port is used to make a connection between the XCell ATF 6 Single-use Device and a bioreactor using the A2B tubing sets. The bottom port on the feed side, at the liquid hemisphere, is the condition/drain port. The top-permeate port is used for harvesting. Connecting a pressure transducer to the top permeate port is recommended to continuously monitor the filter performance during cell-culture processes. Using both permeate ports does not yield any additional benefits. As a result, the bottom permeate port remains clamped.

Figure 3. XCell ATF 6 Single-use Device Ports and Stainless-Steel Stand



### 5.4 Connecting XCell ATF 6 Single-use Device to a Bioreactor

Two types of connections between the device and bioreactor are available. Hard connections are used where steam-in-place (SIP) is an option, such as with stainless steel bioreactors (SSB). Soft connections are used with single-use bioreactors (SUB) and consist of single-use connectors such as AseptiQuik.

Fluid management for XCell ATF 6 Devices include retentate (A2B) tubing set kits and accessories that connect the device with the bioreactor, ensuring proper exchange of cell culture material. Intended for use in pilot scale, clinical, and commercial bioprocessing environments, tubing set kits work with XCell LS Controllers and legacy C410 controllers. Additional permeate tubing (not provided) is required to connect the device to the harvest vessel and should be sterilized by autoclave or disposable sterile coupling. The permeate tubing should be compatible with the filtrate/harvest pump.

**Note:** Bioreactor adapters, used to connect tubing set kit to the bioreactor, may need to be used based on the bioreactor configuration, and are not provided.

#### 5.4.1 XCell ATF 6 Tubing Set Kits

The tubing set kits (Figure 4. XCell ATF 6 Device with Tubing Set Kit) are designed to ensure that the ratio of pump displacement volume to device and A2B hold up volume is greater than or equal to one at the minimum and maximum ATF flow rate. Meaning that the Displacement to Dead Volume Ratio (DDVR) is  $\geq 1$ .

A2B tubing set kits are available in multiple configurations based on the type of bioreactor in use and the desired aseptic connector format (Table 4, Table 5, Table 6). In addition to the tubing set kits, accessories are required (Table 7, Table 8).

Each tubing set kit contains multiple components which enable the complete connectivity and use of the XCell ATF Single-use device. Tubing set kits also include external tubing pinch clamps for isolating flow paths and sanitary clamps for securing aseptic connections. All tubing components are constructed of platinum-cured silicone and are configured with vent filters and aseptic connectors. All tubing set kits are sterilized by gamma irradiation and packed in double plastic bags to maintain sterility.

**Notes:** Aseptic connectors must be linked to a mating connector for use. Unlinked aseptic connectors will leak. To maintain system sterility, extra care must be taken during setup to not to introduce liquid onto the connector.

*Although superficially similar, the tubing sets for the different devices are not interchangeable. Check the labeling on your tubing set to make sure it is appropriate for your device.*

*Flow sensors are developed and calibrated for specific tubing geometries and MOCs. Thus, for intended ATF performance, the flow sensors must only be used with Repligen-supplied tubing set kits.*

*The tubing set kits, permeate pressure sensor kits, and flow sensors are ordered separately. While both the LS and C410 controllers are compatible with the tubeset kits, the LS controller is the only one that offers the flow sensor feedback option. Refer to the respective controller guides for additional details.*

**Table 4. XCell ATF 6 Single-use Device Tubing Specifications**

Item	Component	Sterile Connector Type	Tubing Specifications		
			ID	OD	Length
Tubing set kit	A2B	AseptiQuik L	$\frac{3}{4}$ "	1.125"	24"
	Top permeate	AseptiQuik G	$\frac{3}{4}$ "	1.125"	12"
	Bottom permeate	AseptiQuik G	$\frac{3}{4}$ "	1.125"	6"
	Sample port/Drain port	AseptiQuik G	$\frac{1}{2}$ "	$\frac{3}{4}$ "	6"
	End cap	AseptiQuik G	-	1.5"	-
Permeate pressure sensor kit	Pressure sensor	AseptiQuik G	$\frac{3}{4}$ "	N/A	4"

Table 5. XCell ATF 6 Connection Kit Details

Part	Description	Purpose
Sanitary tri-clamps	1.5" clamps	Clamp connectors
ATF to Bioreactor Connection	Tee with vent filter	Connect to bioreactor, pre-use wetting and integrity testing
ATF to Bioreactor Connection	A2B line	Connect to bioreactor
Permeate Connection	Top permeate (optional pressure sensor kit)	Connection to top permeate port, pressure monitoring (if included), pre-use wetting, and integrity testing
Bottom permeate extension	5" tubing with end cap	Clamp off bottom permeate
Drain connection	Drain tee	Connect to drain port, wetting, draining

Figure 4. XCell ATF 6 Device with Tubing Set Kit

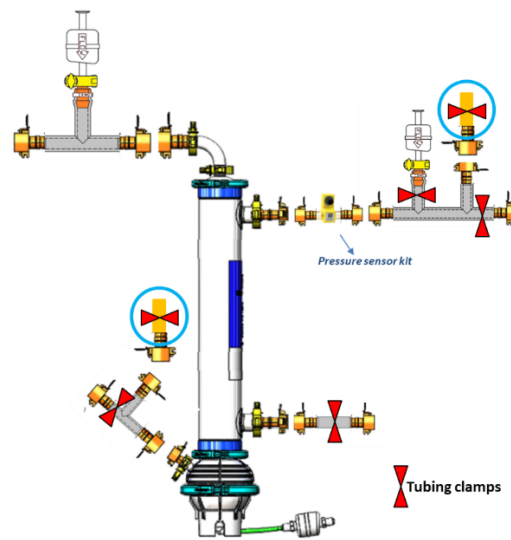


Table 6. Tubing Options

Tubing Set Part Number	Bioreactor Type	Tubing Connection	Included Components
SUATF6-TSK-AQGL-M1	Single-use and Stainless steel	AseptiQuik	A2B, vent tee, top and bottom permeate, drain

The schematic below depicts the components and accessories for the SUATF6-TSK-RM tube set kit, including tubing connections, clamps, and bio-clamps. Depending on the connection requirements, part numbers SUATF6-TSK-AQGL-M1 and SUATF610-PSK-AQG-V2 will have corresponding accessories and components.

Figure 5. Tube Set Kit Components and Accessories

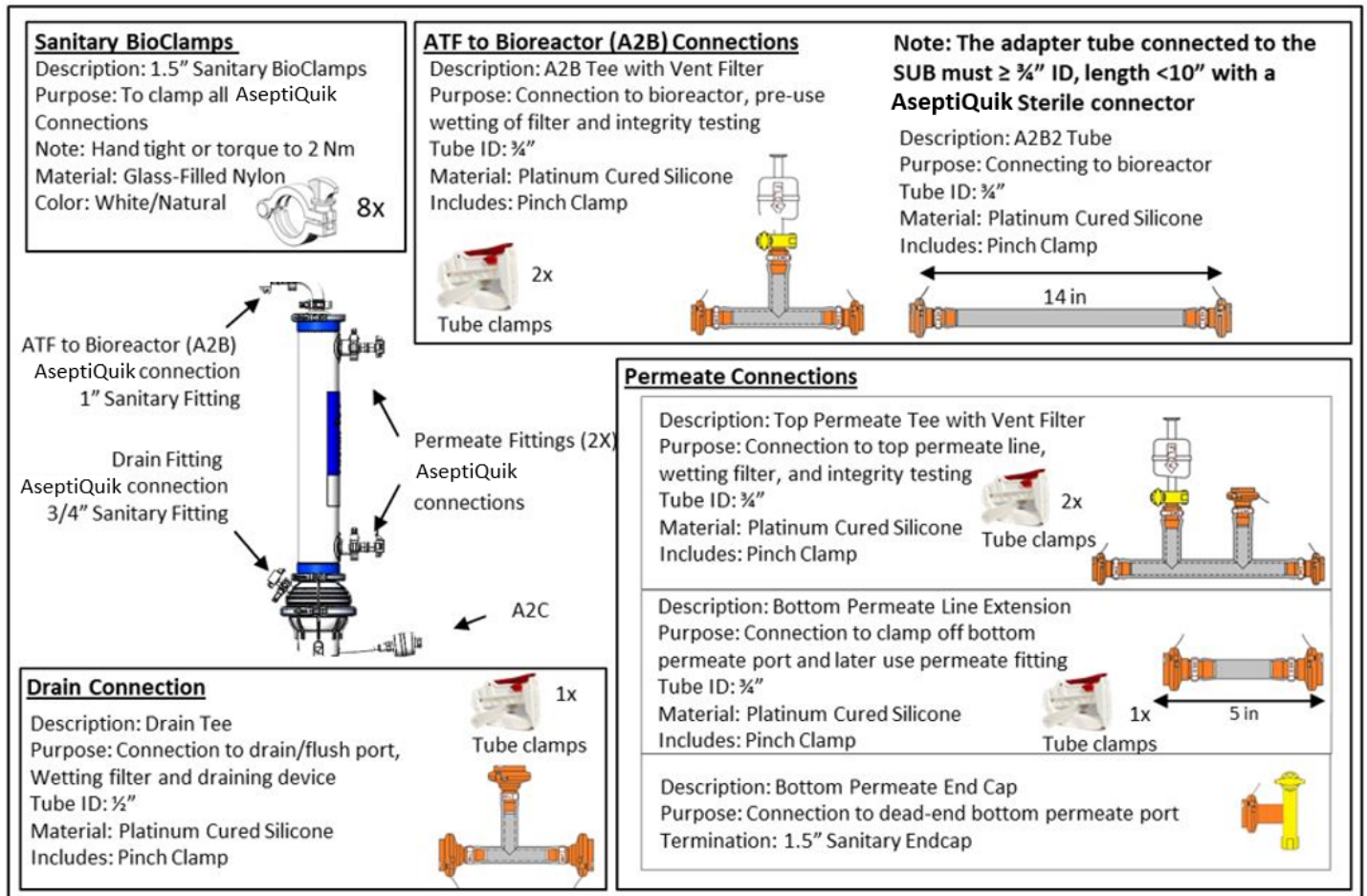


Table 7. Required Accessory

Description	Part Number	Aseptic Connector	Single or Multi-use	Pressure Rating	ID (inches)	Material of Construction
Permeate Pressure Sensor	SUATF610-PSK-AQG-V2	AseptiQuik	SU	25 psi	3/4"	Polycarbonate

In addition, use of a SSB requires a bivalve assembly and connector, such that the XCell ATF 6 Single-use Device can be connected to the SSB through a sterile connection.

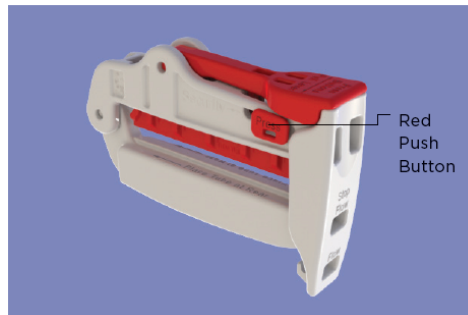
**Table 8. Required Stainless Steel Bioreactor Accessories**

Description	Part Number	Tubing Connection	Single- or Multi-use	Pressure Rating	ID	Material of Construction
Bivalve assembly	ATF6-VLV-KIT	N/A	MU	45 psi	3/4"	Stainless steel
Connector	SUATF6-AQG-TO-0.75TC	AseptiQuik	SU	25 psi	3/4"	Polycarbonate

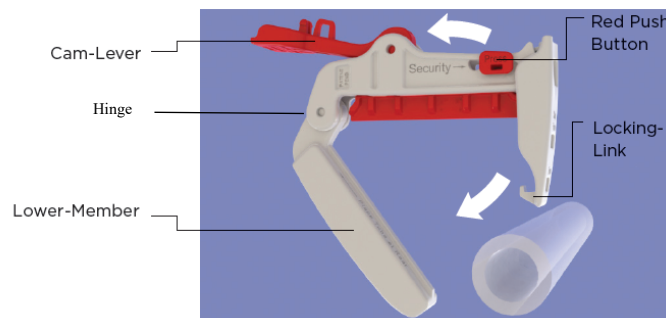
#### 5.4.2 Proper Use of External Tubing Clamps

The XCell ATF 6 Tube Set Kits are supplied with Pure-Fit® TCL clamps. These clamps are placed externally on tubing segments and are used to isolate specific tubing segments during pre-use wetting and operation of the device. It is important that the Pure-Fit TCL clamps are oriented and clamped correctly on the tubing segments to ensure complete clamping or closure of the tubing segment. Operation of the Pure-Fit TCL is as follows:

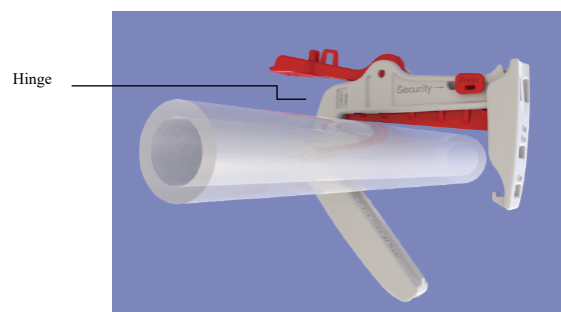
1. Starting from the closed position, simultaneously press and hold the two red push buttons.



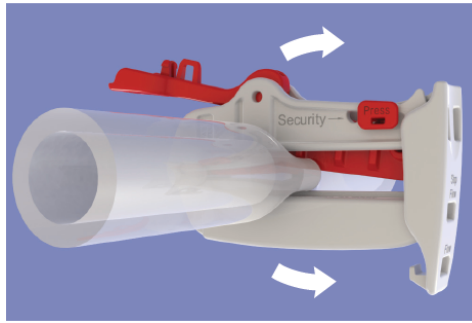
2. While pressing the buttons, pull the cam lever back toward the hinge of the clamp.
3. Pull the lower member away from the locking ring.



4. Position the tube in the gate, as close as possible to the hinge of the clamp.



5. Push the lower member to the desired position (Flow or Stop Flow).



6. Rotate the cam lever to the closed position. The tubing is now held in the Flow position or locked in the Stop Flow position (shown).



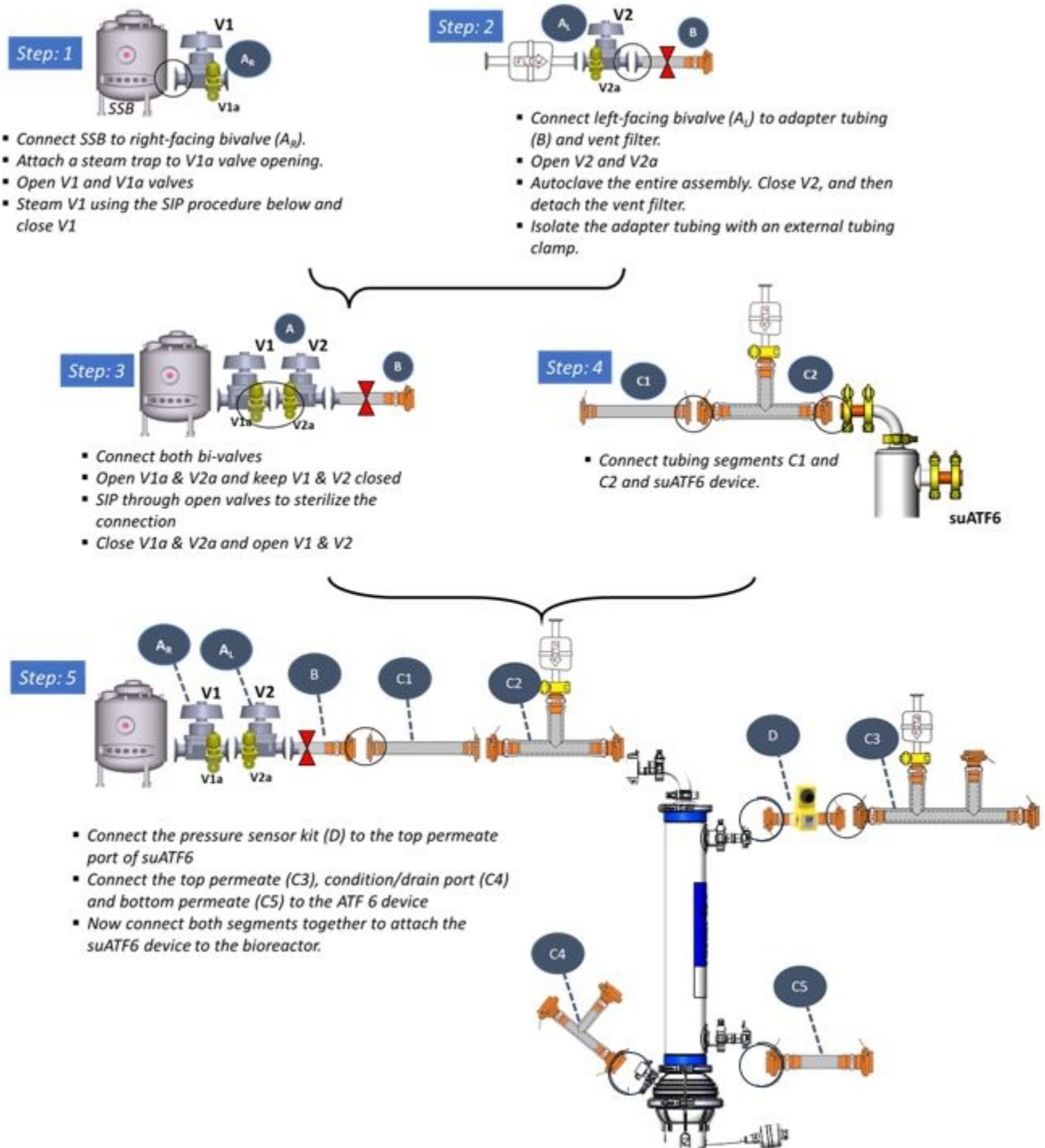
#### 5.4.3 Tubing Set Kit Assembly: Single-use XCell ATF 6 Connected to a Stainless-Steel Bioreactor

Tubing set kits are available with AseptiQuik connectors. Hard connection between the tubing set and the stainless-steel bioreactor consists of the following parts, available as a unit:

- Two isolation bivalves: one attached to the bioreactor, and one attached to the component “B”.
- A flexible hose between the main branches of the two valves
- A steam inlet: a side valve on one bivalve
- Condensate drain: a side valve on the other bivalve; lowest of the available valves

Instructions for connecting the tubing set to a stainless steel bioreactor are provided in [Figure 6](#).

Figure 6. Single-use XCell ATF 6 Connected to a Stainless-Steel Bioreactor



- A. ATF6-VLV-KIT (bivalve).
- B. SUATF6-AQG-TO-0.75TC (tubing set).
- C. SUATF6-TSK-AQGL-M1 (tubing set).
- C1. A2B tubing.

- C2. Vent tee.
- C3. Top Permeate tubing.
- C4. Drain/condition tubing.
- C5. Bottom permeate tubing.
- D. SUATF610-PSK-AQG-V2 (pressure sensor kit).

**Connection Instructions:**

1. Connect the right facing bivalve ( $A_R$ ) to the stainless-steel bioreactor.
2. Attach the steam trap to the opening at V1a. Open valves V1 and V1a.
3. Steam the V1 valve per the required steam-in-place (SIP) procedure to sterilize the bioreactor connection. A general SIP protocol is provided below.
4. After completing the SIP procedure, close V1.
5. Connect the left facing bivalve ( $A_L$ ) to tubing set (B) that is attached to the XCell ATF 6 Single-use Device.
6. Open Valves V2 and V2a.
7. Autoclave the entire assembly. Close V2.
8. Isolate the adapter tubing with an external tubing clamp and connect the two halves of the bivalve.
9. Ensure that V1a and V2a positions are open and that V1 and V2 positions are closed.
10. Steam the bivalve connection through the open valves per the required SIP procedure.
11. After completing the SIP procedure, close V1a and V2a and open V1 and V2.
12. Connect tubing segments B, C1 and C2 to each other.
13. Connect the free end of the C2 tubing set to the XCell ATF 6 Single-use Device.
14. If using, attach the pressure sensor kit (component D) to the top permeate port on the XCell ATF 6 Single-use Device.
15. Attach the condition/drain port (C3) and bottom permeate extension (C4) to the XCell ATF 6 Single-use Device.

The following general steam-in-place procedure is recommended for making non-single use connections. End-users should utilize their SIP procedures and Safety protocols.

1. Attach a steam trap to the condensate valve and connect to a drain.
2. Close steam inlet and condensate drain valves.
3. Securely connect a regulated steam source to the steam inlet valve.
4. Open steam service.
5. Slowly open steam inlet valve.
6. Slowly open steam condensate valve.
7. Sterilize the connection for 20 minutes at 121°C and 16 psi.
8. Cool down.
9. Close the condensate valve.
10. Rapidly close the steam inlet valve and immediately open the main valve into the filtration assembly to minimize a pressure drop in the connection.

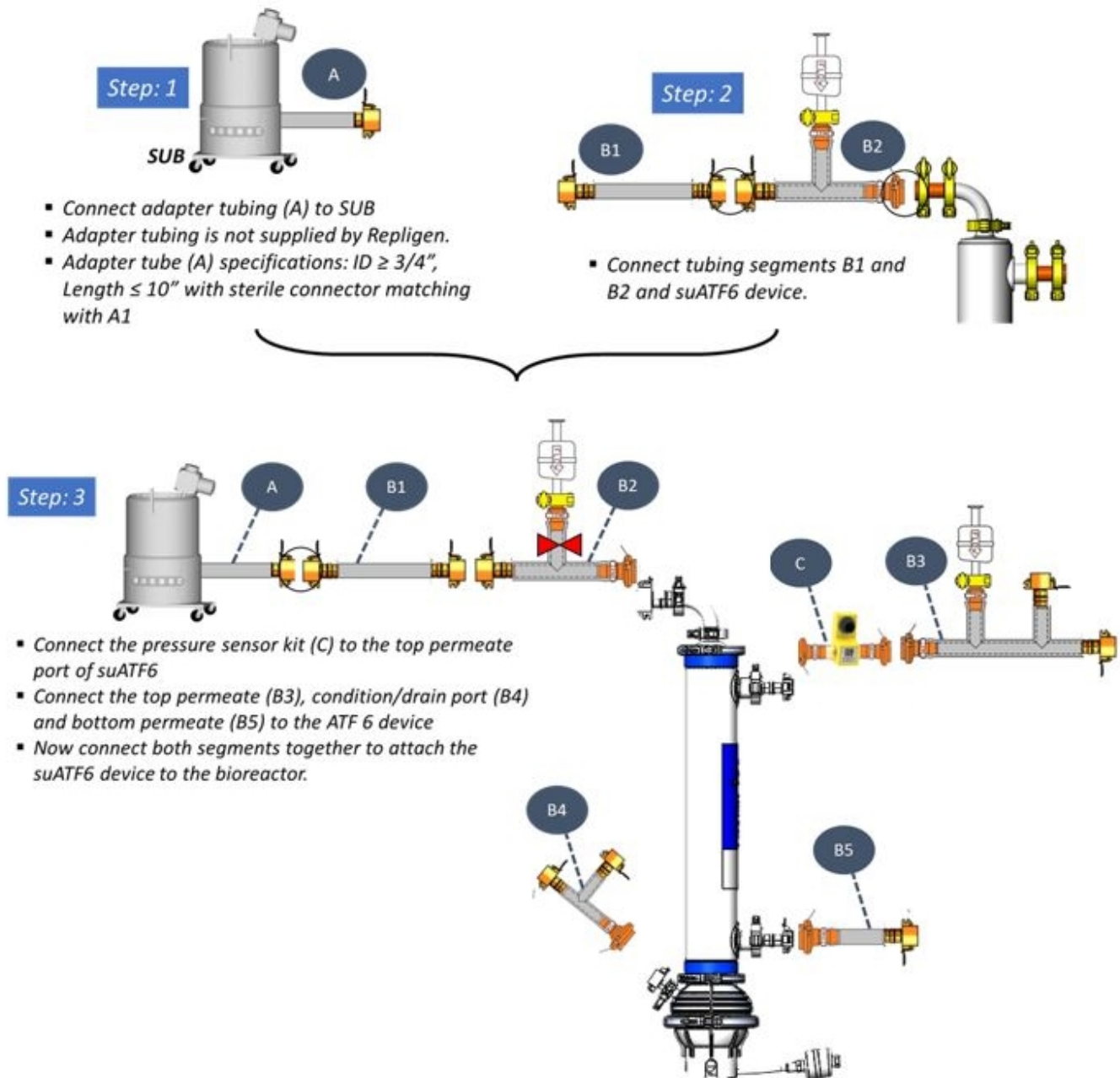
#### 5.4.4 Tubing Set Kit Assembly: Single-use XCell ATF 6 Connected to a Single-use Bioreactor

Single-use XCell ATF 6 Tube Set Kits for use with single-use bioreactors are available with AseptiQuik (AQG or AQL) connectors. The schematic presented below depicts only the AseptiQuik AQG connector; however, the same applies to other sterile connectors.

Instructions for connecting the tubing set to a single-use bioreactor are provided in [Figure 7](#).



Figure 7. Single-use XCell ATF 6 Connected to a Single-use Bioreactor



- A. Bioreactor adapter tubing (Not provided).  
 B. SUATF6-TSK-AQGL-M1 (tubing set).  
   B1. A2B tubing.  
   B2. Vent tee.  
   B3. Top permeate tubing.  
   B4. Drain/condition tubing.  
   B5. Bottom permeate tubing.  
 C. SUATF610-PSK-AQG-V2 (pressure sensor kit).

Tubing Set Connection Instructions:

1. Connect adapter tubing (not provided) to SUB.

2. Connect tubing set (A1) to adapter tubing and vent tee (A2).
3. Connect vent tee (A2) to XCell ATF 6 Device.
4. Attach pressure sensor kit (B) to top permeate port.
5. Attach condition/drain port (A3) and permeate extension (A4).

## 5.5 XCell ATF 6 Single-use Device Wetting

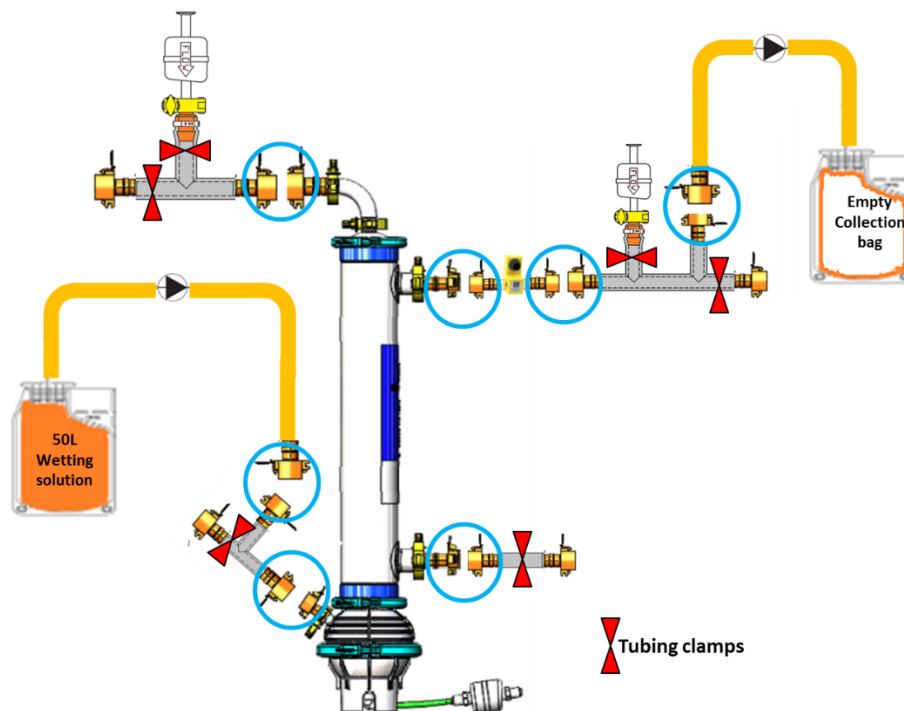
### 5.5.1 Device Wetting Background

The XCell ATF 6 Single-use Device is supplied dry and gamma irradiated. Wetting of the hollow fiber filter is required to ensure robust filter performance. Wetting can be performed either offline, not attached to the bioreactor, or online, attached to the bioreactor. Offline wetting does not require the use of the XCell LS Controller or C410 Controller and allows for pre-use filter integrity testing while maintaining sterility.

The online wetting procedure is executed with the device connected to a bioreactor that contains sterile cell culture media prior to inoculation. The XCell LS Controller is capable of wetting 2x suATF6 devices simultaneously, while the C410 Controller can only wet one device at a time.

### Offline Wetting

Figure 8. XCell ATF 6 Single-use Device Offline Wetting Setup



Instructions:

1. Install the device into the stand and attach the tubing per the configuration shown in Figure 8;

**Note:** Required device stand not shown.

2. Create AseptiQuik connections as shown in above figure (Figure 8), and match AseptiQuik tab orientations. This will allow fluid movement for the offline wetting purposes. Ensure AseptiQuik installation is done correctly per manufacturer recommendation.

**Note:** Figure 8 features one sterile connector.

3. Ensure external tubing clamps are placed in the proper locations per the configuration shown in [Figure 8](#), for effective filter wetting and to prevent the accidental wetting of the vent filters and unlinked aseptic connectors.
4. Connect a 50 L bag containing sterile wetting solution to the condition/drain port.
5. Connect an empty 50 L bag to the required connection on the top permeate port.
6. Pump wetting solution through the condition/drain port at a flow rate of 1 LPM to fill the device.
7. Stop the pump when the solution begins to fill the top permeate port. Clamp the top permeate port completely such that both the vent filter and empty bag segments are closed.
8. Restart the pump briefly. When solution begins to fill the elbow on at the top of the device, stop the pump and clamp the vent filter segment to close the top retentate completely.

**Note:** Ensure that the wetting solution does not contact the vent filters or the unlinked aseptic connectors at any point during the wetting process. Add an external tubing clamp to the top permeate tubing set to isolate (close) the vent filter line, leaving the line to the collection bag open.

9. Restart the pump at a flow rate of 3 – 4 LPM, allowing for a minimum wetting time of 15 minutes. Continue until the entire volume of wetting solution has passed through the device and into the permeate collection. It is recommended to tilt the device until the air above the top permeate port is removed. Stop pump.
10. Clamp feed and collection tubing to remove bags from device.
  - a. If cell culture media was used for wetting, the media collected in the permeate bag can be used to assess the sterility of the device by incubating it in a shake flask.
  - b. The wetting solution can remain inside the device until the unit is ready to be connected to the bioreactor. Ensure that tubing lines to the vent filters on the top retentate and top permeate tubing sections are clamped.

### 5.5.2 Filter Integrity Evaluation

If filter integrity testing is required, the recommendation is that it be performed following the offline wetting step and prior to connection to the bioreactor. The wetting solution must be drained from the device before testing.

1. Remove the clamps at the vent filters on both the top retentate and top permeate ports.
2. Close the line to the collection bag with a clamp and ensure that the condition/drain port clamp is correctly positioned ([Figure 8](#)).
3. Start pump in reverse flow at flow rate of no more than 0.5 LPM to drain the wetting solution into the bag at the condition/drain port.

**Note:** The draining process will not remove all of the solution from the diaphragm pump. The presence of residual wetting solution in the pump will not affect the filter integrity evaluation or the functionality of the device.

4. After draining the wetting solution, stop the pump and disconnect the bags from the device in a sterile manner by clamping the respective tubing lines.
5. The device integrity can now be evaluated using a forward air diffusion test by connecting a FIT Tester to the vent filter on the top retentate tubing set.
6. Ensure that the vent filter on the top permeate tubing is open for diffused air to escape and all remaining pinch clamps are tightened to avoid leaks ([Figure 9](#)).
7. Pressurize the unit through the top retentate port vent filter to 10 psi and monitor the pressure decay for five minutes. The average pressure decay should not exceed 0.5 psi/min (diffusion rate <30 SCCM/m<sup>2</sup>) for the unit to pass the filter integrity test.

**Note:** To avoid filter drying, it is recommended to clamp the segments leading to vent filters on both permeate and retentate side after FIT evaluation.

Figure 9. Configuration for Draining the Wetting Solution Following Offline Wetting

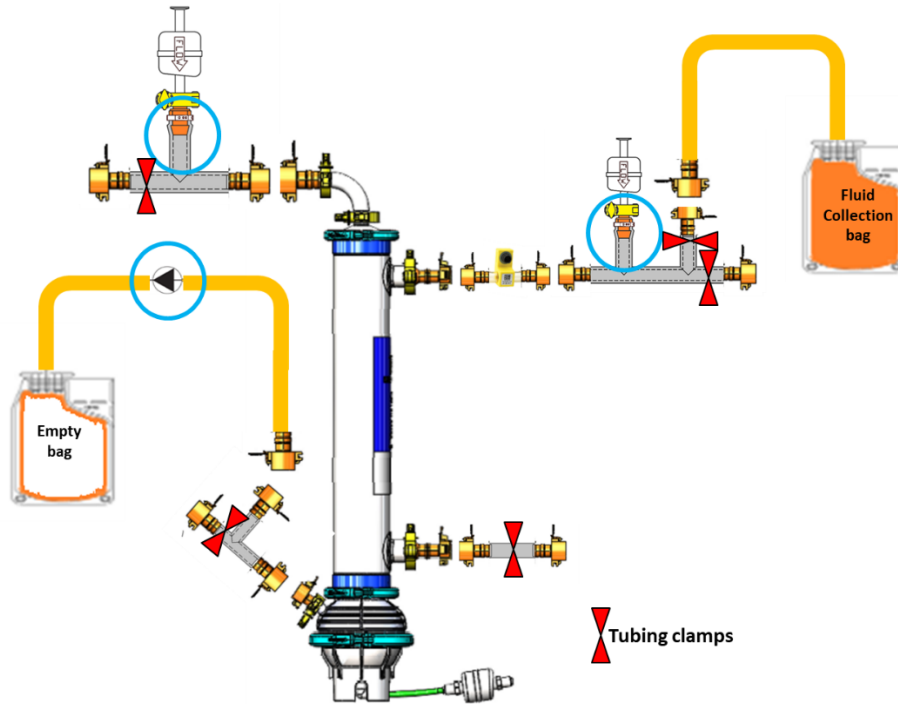
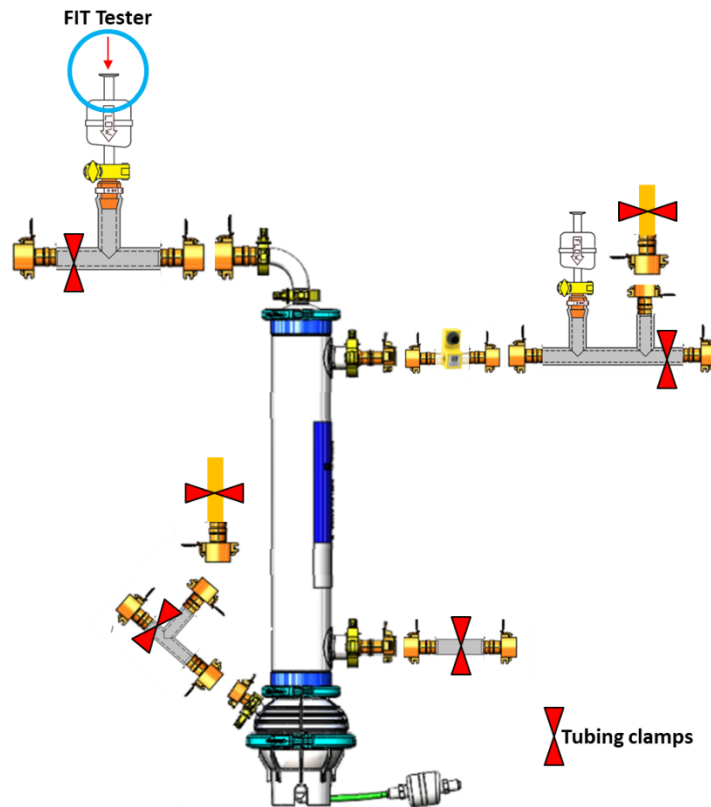


Figure 10. Configuration for Post-wetting Device Integrity Testing



### 5.5.3 Connecting to a Bioreactor

After completing the offline wetting procedure and/or the integrity test, use the A2B tubing set to make a connection between bioreactor and the device by using a sterile connector or a tube welder.

Refer to the XCell LS Controller user guide or C410 Controller User Guide for instructions on operating the XCell ATF 6 Single-use Device.

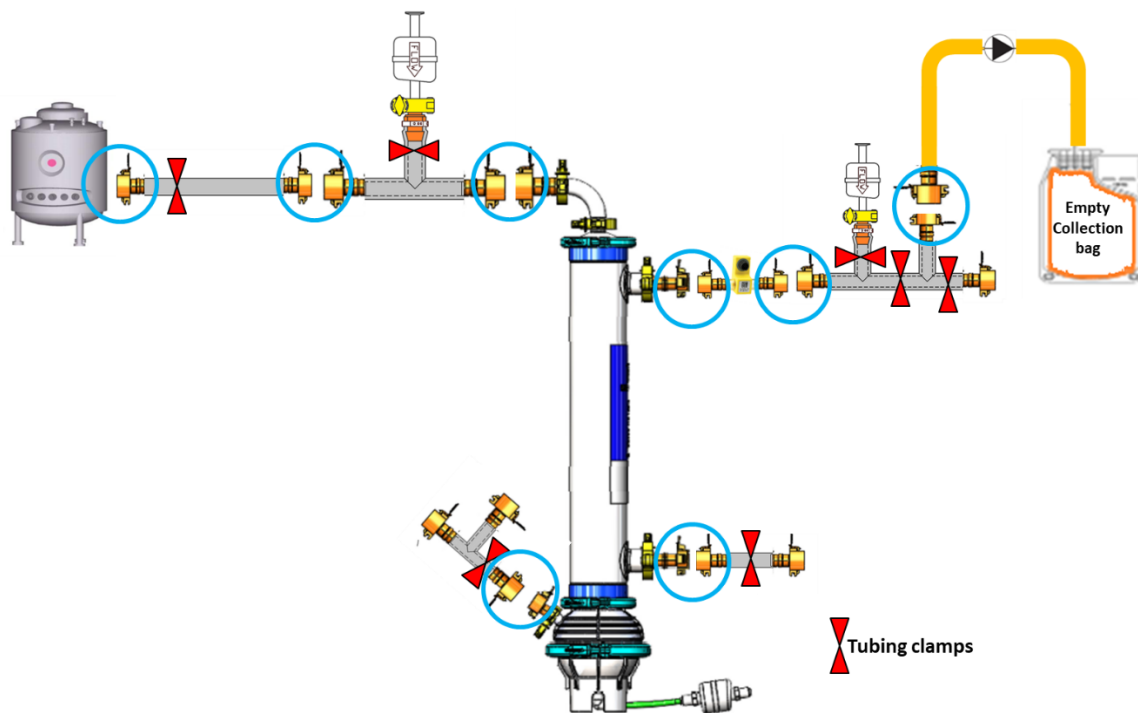
### 5.5.4 Online Wetting

If not wetted offline, after connecting to the bioreactor, devices are wetted using the XCell LS Controller or C410 Controller. This method is not amenable to performing a pre-use integrity test.

Instructions:

1. Install the device into the stand and attach the tubing ([Figure 11](#); stand not shown).
2. Connect aseptic connectors (circled). Ensure tubing clamps are placed in the proper locations for effective filter wetting and to prevent the accidental wetting of the vent filters and unlinked aseptic connectors.
3. Connect the device to the bioreactor using the A2B tubing set.
4. Connect the controller to the device via the A2C connection.
5. Connect an empty 50 L bag to the top permeate port.
6. Open clamps on retentate tubing segment ensuring the line between the bioreactor and device is open. Clamp off the vent filter.

**Figure 11. Configuration for Online Wetting of the Device (Pre-wetting)**



**Note:** Bioreactor head pressure may force media into the device without controller action.

1. Start the XCell ATF Device pump from the XCell LS Controller or C410 Controller at a flow rate of 17.2 LPM (pressure and exhaust) and let it run until the flow set-point is reached.
2. After equilibration, remove the clamp to the empty bag on the permeate port and immediately start permeate pump at a flow rate 3 – 4 LPM.

3. Collect at least 50 L of wetting solution in the collection bag (Figure 12). The flow rate should be adjusted to allow for a minimum wetting time of 15 minutes.
4. Stop the permeate pump and then the XCell ATF Device pump. Clamp the A2B tubing line and disconnect the collection bag from the top permeate port in a sterile manner by clamping the tubing (Figure 12). The remaining solution inside the device remain until the bioreactor is ready for the perfusion process.

**Note:** Close the retentate and permeate vent filters before starting the perfusion process.

Figure 12. Configuration for Online Wetting of the Device (Post-wetting)

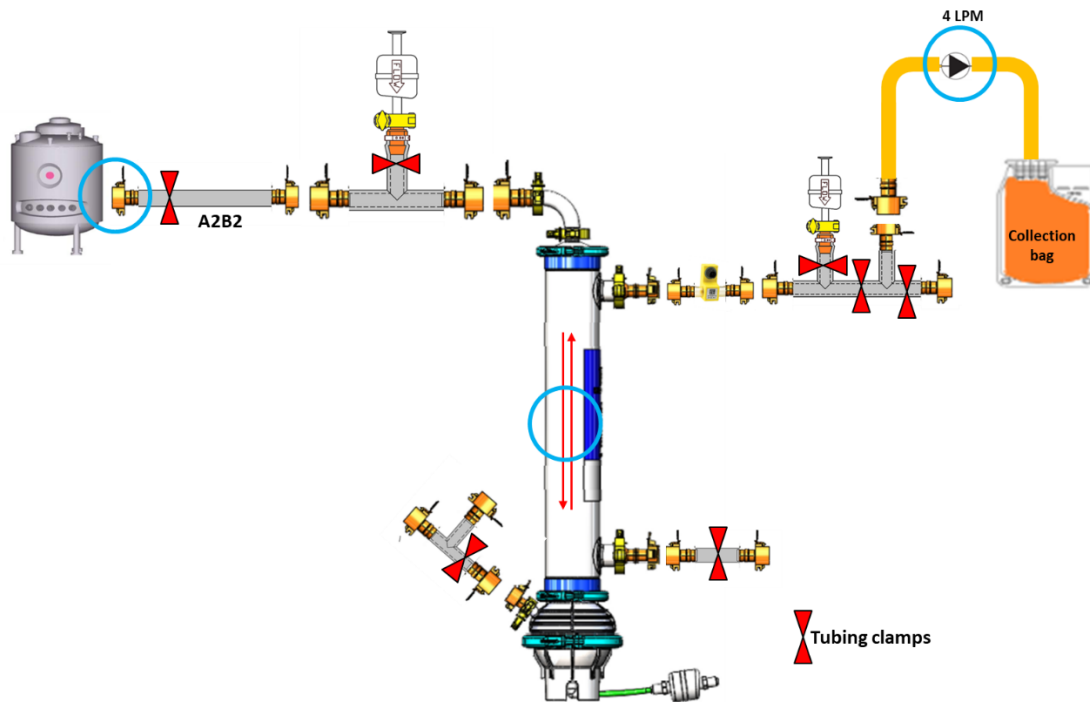
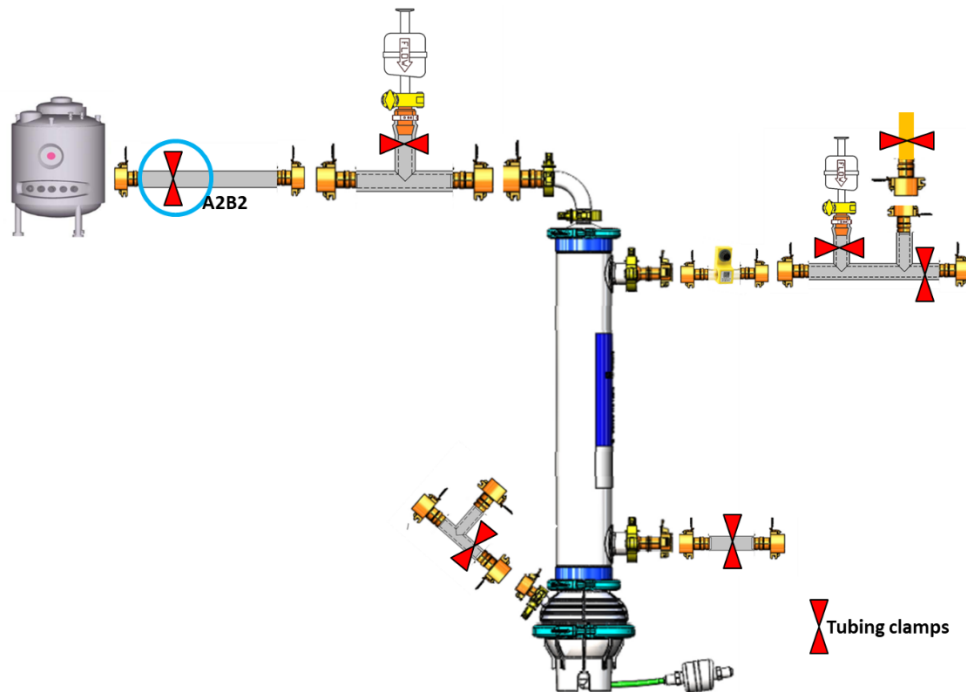


Figure 13. Clamp Configuration for Disconnecting Bags from Device



### 5.6 XCell ATF 6 Single-use Device Post-use Instructions

After completion of a cell culture process using the XCell ATF 6 Single-use Device, disconnect, drain, and discard the device. Specific disposal procedures will be end-user dependent. The below instructions are provided as a general guideline.

#### Instructions:

1. Stop the permeate pump and disconnect the harvest bag in a sterile manner.
2. Stop the ATF Controller and disconnect the XCell ATF 6 Single-use Device A2C line from the controller.
3. Clamp both the A2B and top permeate tubing lines using tubing clamps.
4. Prepare an empty bag or a container (>5 L) fitted with an Aseptiquik connector. Connect the empty bag to the condition/drain port.

**Note:** Ensure that the tubing between the drain port and the empty bag is at least 2 feet long to attach a pump.

5. Remove the clamps at the vent filters on both the top retentate and top permeate ports. Detach the clamp at the condition/drain port to drain the culture into the empty bag.
6. Connect a pump to the drain port and begin the draining of cell culture solution at a flow rate of 1 LPM to the bag/container.

**Note:** The device may need to be tilted to drain the cell culture solution from the diaphragm pump.

7. After draining, stop the pump and disconnect the bag from the device. Now the device is ready to be discarded by following proper waste disposal codes.

## 6. Frequently Asked Questions

### What do I do if a vent filter accidentally becomes wet during the device wetting process?

Vent filters are made of a hydrophobic membrane and are designed for the sterile filtration of gas. Getting wetting solution in contact with vent filters for short duration (<15 minutes) does not impact the integrity nor sterility of vent filter and XCell ATF 6 Single-use Devices. We recommend purging the vent filter to remove the residual solution.

### What do I do if a leak is detected during the off-line wetting procedure?

At point of manufacture, each individual device is pressure tested at 25 psi to ensure the integrity of the entire assembly. However, if a leak is detected during the wetting process, immediately stop the peristaltic pump and identify the location of the leak. Please ensure that the sterile connectors, required sanitary clamps and external tubing clamps are appropriately installed at the proper, required locations. Clamping the wrong tubing sets during the wetting procedure could result in over-pressurization of the device and leakage at non-integral positions. If no faults were found in setup, please contact a local FAS or customer service for further support.

### How do I ensure the sterility of an XCell ATF 6 Device?

The wetting solution collected from the permeate during the off-line wetting procedure can be incubated in a shake flask at 37° C for 24 hours to assess the sterility of a device.

### How long can the XCell ATF 6 Single-use Devices be stored in a wet condition before connecting to a bioreactor?

After completing the procedure, the tubing segment leading to the vent filters on the A2B and the top permeate tubing segments must be clamped to ensure the hollow fiber filter remains wetted and does not dry out. The device can be stored in a wet condition for one week in water or cell culture media before installing the device for cell culture processing. The device cannot be stored in ethanol, caustic solutions or acidic solutions nor should bleach be used to sanitize.

### What do I do if the device fails pre-use integrity testing?

If a pre-use integrity failure is realized, please ensure that the external tubing clamps on the retentate side are properly installed. Also, ensure that the sterile connectors and any accompanying clamps are installed and securely tightened. Generally, if the filter integrity test fails by small percentage (acceptance criteria: 30 SCCM/m<sup>2</sup>), it is recommended to wet the filter again using the same procedure. If a gross leak is detected during integrity, please contact a local Account Manager or Customer Service for further support.

### Do the XCell ATF 6 Devices perform similarly to XCell ATF 6 Stainless Steel Devices?

Yes, the filter used in the XCell ATF 6 Single-use Devices is same as the one which is being used in the XCell ATF 6 Stainless Steel Devices. In addition, the ATF pump configuration is the same between the single-use and stainless-steel product formats and both formats can be operated with the XCell LS Controller and C410 Controller.

## 7. Appendix A: Part Numbers and Materials of Construction

Table 9. XCell ATF 6 Single-use Device, Tube Set Kit and Accessory Part Numbers

Part Number	Description	Notes
SUATF6-R02P-A	XCell ATF 6 Single-use Device, 0.2 µm PES	0.2 µm filter, CPC AseptiQuik aseptic connections
suATF6-STD	XCell ATF 6 Single-use Device Stand	Stainless steel reusable stand
SUATF6-TSK-AQGL-M1	XCell ATF 6 Single-use Device Tubing Set Kit	(6) Individual tubing sets (8) Sanitary BioClamps (6) Pinch Clamps
SUATF610-PSK-AQG-V2	XCell ATF 6 Single-use Device Permeate Pressure Sensor Kit	(1) ¼" TC SciLog SciPres Gen II Pressure Sensor (2) AseptiQuik ¼" TC Connectors



## 8. Materials of Construction

**Table 10. XCell ATF 6 Single-use Device Materials of Construction: Product-contact Parts**

Component	Materials of Construction
Filter housing and pump	Polycarbonate
Adhesive	Acrylated Urethane
Tubing	Platinum Cured Silicone
Pressure sensor	Polysulfone, Silicone, Polycarbonate
Elbow	Polyvinylidene Fluoride (PVDF)
AseptiQuik G and L Connectors	Polycarbonate with silicone seal
Hollow fiber cartridge	PES Membrane - Polyethersulfone, Polysulfone, Polyurethane, and Polypropylene
Gaskets and diaphragm	Silicone

**Table 11. XCell ATF 6 Single-use Device Materials of Construction: Non-product-contact Parts**

Component	Materials of Construction
Tubing set non-product-contact materials	Glass-Filled Nylon clamps, nylon cable ties, stainless steel tubing clamps, polypropylene end caps, polypropylene air filters with PDVF outer capsule
Air side tubing and fittings	Polyurethane tubing, PVDF fittings
Stand	ABS, SS

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