SHORT PROTOCOL No. 56

Setup and Use of Packed-bed Bioreactors with Fibra-Cel® Disks and the BioFlo® 320 Bioprocess Control Station

Min Tang-Schomer, Amanda Suttle, and Ma Sha
Eppendorf Inc., Enfield, CT, USA
Contact: bioprocess-experts@eppendorf.com

Abstract

This protocol describes the detailed procedure of the setup and use of a packed-bed Fibra-Cel® Disk bioreactor controlled by a BioFlo 320® control station. Fibra-Cel Disks are proven support systems used to increase yields of anchorage-dependent cell cultures. When combined with the Eppendorf packed-bed impeller system, the Fibra-Cel Disks create a three-dimensional matrix that immobilizes cells within the bioreactor. This immobilization also protects cells from damaging shear forces, which helps to prevent cell loss and achieve high cell densities.

We guide the users through all steps of the bioprocess, starting from setting up the packed-bed Fibra-Cel bioreactor to the preparation and operation of a perfusion culture. Furthermore, we provide application examples for packed-bed Fibra-Cel bioreactors demonstrating high cell densities for various cell types, including CHO, hybridoma, Vero and HEK 293T cells. This short protocol will be useful for those who are new to packed-bed bioreactors and Fibra-Cel Disk-based perfusion cultures.

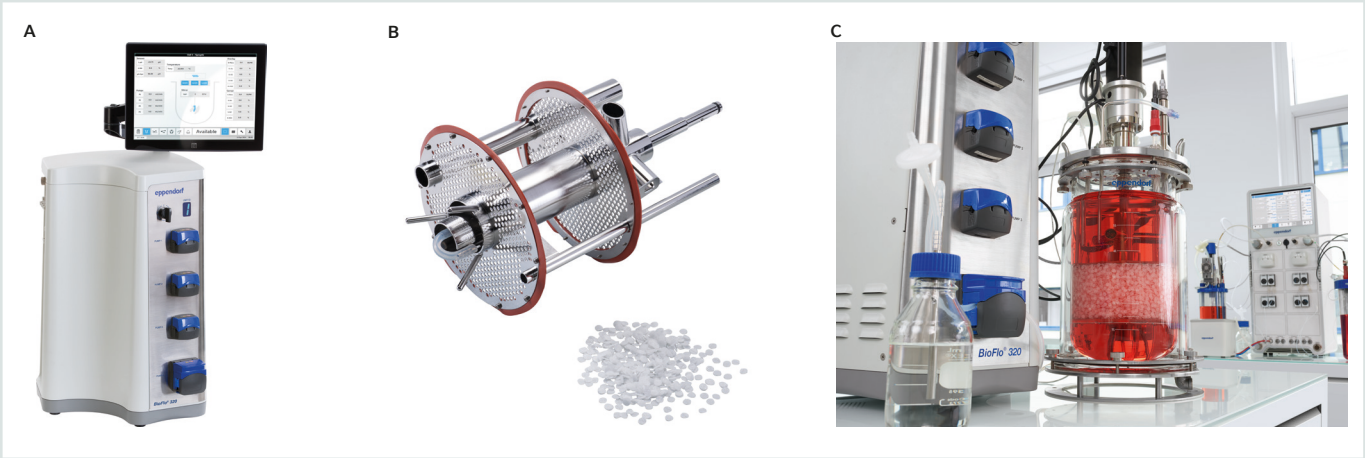

Figure 1: **(A)** The BioFlo 320 bioprocess controller. **(B)** Packed-bed impeller and Fibra-Cel Disks for cell attachment. **(C)** Glass bioreactor equiped with a Fibra-Cel Disk-filled packed-bed impeller under control of the BioFlo 320 bioprocess controller.

Learn more about of the BioFlo 320 bioprocess controller at: www.eppendorf.com/bioflo320

Content

Introduction

Cells are cultured either in suspension or adherent to biomaterials such as microcarriers or synthetic scaffolds. Adherent cell cultures remain necessary for many mammalian cells, including stem cells used for cell and gene therapy. For bioreactor-based scale-up cultures, it is important to design strategies for sterile incorporation of synthetic scaffolds in the bioreactor, efficient cell adherence to the scaffold and achieving high cell density.

A bioreactor with a packed-bed impeller can address the first two needs by combining high-surface area Fibra-Cel Disks and fluid dynamics-driven directional flow. The BioFlo 320 bioprocess control station can provide comprehensive and easily-adaptable control for bioreactor cultures. Thus, use of a packed-bed bioreactor with Fibra-Cel Disks under control of a BioFlo 320 bioprocess control station can achieve high cell density of adherent cell cultures.

A Fibra-Cel Disk is a polyester/polypropylene scaffold developed for adherent mammalian cell culture [1]. Packed-bed bioreactors with Fibra-Cel Disks have been used for vaccine production from various cell sources, including Vero and CHO cells [2,3].

Sterile incorporation of Fibra-Cel Disks into a bioreactor system must be ensured during assembly at the very beginning of the process. Since cell removal and counting

cannot be directly performed during the cultivation, cell dissociation from the scaffold and cell counting at the end of the culture process is necessary to obtain cell densities.

In this protocol, we describe the components of a glass bioreactor equipped with a packed-bed impeller and Fibra-Cel Disks, and guide the users through the assembly process. We also present the steps for the preparation and operation of a perfusion culture that helps achieving a high cell yield. We describe the calculation of cell densities from glucose consumption to assist the user with the analysis of samples. Finally, we provide examples of applications that use packed-bed Fibra-Cel Disk bioreactors to achieve high cell densities, encompassing various cell types including CHO, hybridoma, Vero and HEK 293T cells [2–5].

It allows users to achieve quick and easy initial culture success and can serve as a starting point for adaptation to user-specific bioprocess applications.

Material and Methods

1. The glass packed-bed bioreactor system

1.1. Assembly

1.1.1. Components of a glass packed-bed bioreactor

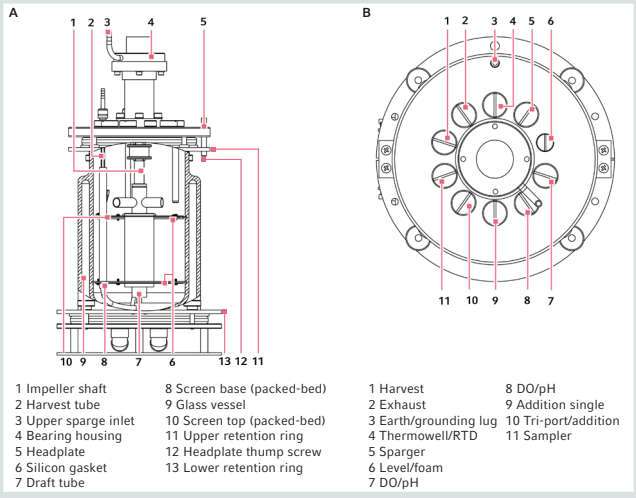

Figure 2: Schematics of a packed-bed bioreactor. **(A)** Bioreactor assembly. **(B)** Headplate.

1.1.2. Attach the impeller shaft to the headplate

> See Figure 3

> Insert the shaft into the bearing housing by aligning the etched notch on the end of the shaft with the etched notch in the locking ring base of the magnetic bearing housing coupling (Fig. 3A-B).

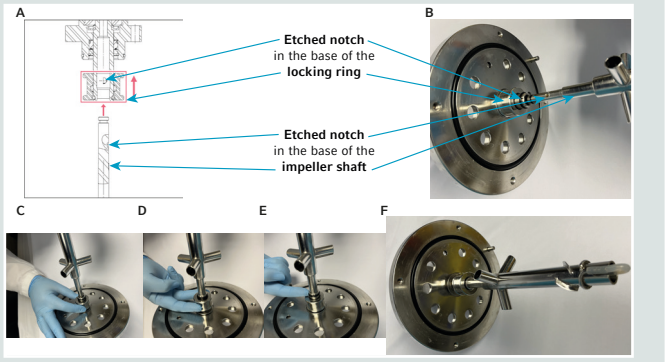

Figure 3: Attach the impeller shaft to the headplate. **(A)** Schematics of the locking ring. **(B)** An example image demonstrating the impeller shaft alignment and insertion. **(C-F)** See text section 1.1.2 for details

> Note: The coupling is located on the underside of the bioreactor headplate.

> After it is aligned, slide the locking ring away from the headplate (magnetic bearing housing) (Fig. 3C), but not completely. Push the impeller shaft into place, while the locking ring is in a middle-position (Fig. 3D). Make sure the notch on the shaft remains aligned with the notch in the locking ring.

> Once aligned properly, the locking ring can be pushed back towards the headplate and the shaft will lock into place (Fig. 3E). In the final position the locking ring is covering the O-ring on the inserted impeller shaft.

> Place the bioreactor headplate with the attached impeller shaft on the lab bench resting the weight of the headplate on the bearing housing to avoid damage to the impeller shaft (Fig. 3F).

> Note: The thermowell, sample tubes, blind plugs, compression fittings for sensors and exhaust condenser will be assembled after the packed-bed is installed and the headplate is resting on the vessel.

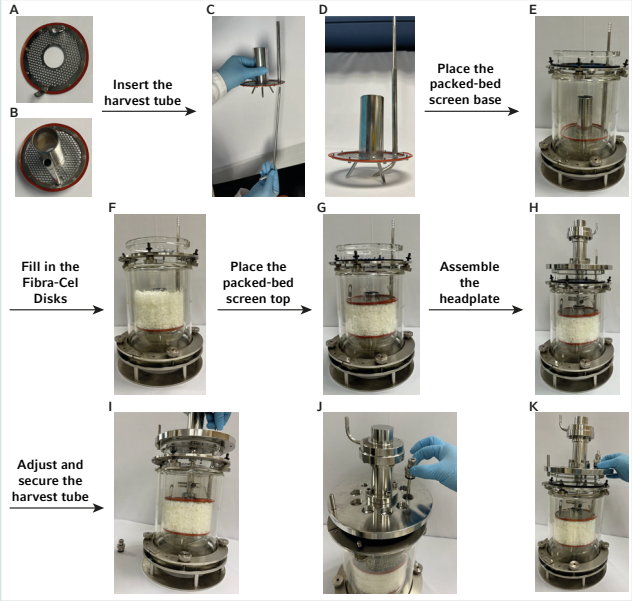

Figure 4: Packed-bed assembly. **(A)** Screen top of the packed-bed. **(B)** Screen base. **(C-H)** See text section 1.1.3 for details. **(I-K)** See text section 1.1.4 for details.

1.1.3. Install the packed-bed into the glass vessel

> See Figure 4

> Insert the harvest tube through the stainless-steel guide tube on the packed-bed screen base before placing it into the vessel (Fig. 4C-D). The harvest tube is the only tube that accesses the liquid below the packed-bed after complete assembly and during a bioreactor run.

> Wet the rubber seal with water and slide the packed-bed screen base into the vessel until stopped by the bottom of the vessel (Fig. 4E).

Table 1: Fibra-Cel Disk quantity for different vessel/packed-bed volumes.

Vessel volume (L)	Packed-bed volume (L)	Approximate quantity of Fibra-Cel Disks to pack (g)	Total 3D surface area (cm^2)
1	0.5	50	6.0×10^4
3	1.5	150	1.8×10^5
5	2.5	250	3.0×10^5
10	5.0	500	6.0×10^5

> Fill the packed-bed with the recommend quantity of Fibra-Cel Disks based on vessel size (Fig. 4F). Take care to avoid the center tube of the packed-bed screen base while filling the Fibra-Cel Disks around into the vessel. The recommended filling density is approximately 100 g/L of packed-bed volume. The packed-bed volume is approximately 50% of the vessel volume (Table 1).

> Place the packed-bed screen top into the vessel (Fig. 4G). Wet the rubber gasket to facilitate the insertion and push the packed-bed screen top all the way down in the vessel till comfortably settled on top of the Fibra-Cel Disks.

> Align the hole in the packed-bed screen top to allow for the harvest tube from the screen base to pass through a free Pg 13.5 port (Fig. 4H).

1.1.4. Assemble the headplate to the glass vessel

> To place the headplate onto the glass vessel, the harvest tube (previously installed) must be lined up with the harvest tube compression fitting on the top of the headplate. To do this, slowly align the harvest tube and compression fitting up while positioning the impeller shaft inside the center tube of the packed-bed screen base.

> Adjust the harvest tube depth to make sure that the internal opening is positioned in-between the bottom of the vessel and the packed-bed screen base (Fig. 4I). Secure the position with the harvest tube compression fitting onto the headplate (Fig. 4J).

> Secure the headplate thumb screws until the headplate is secured to the upper retention ring. Only hand-tighten the thumb screws (Fig. 4K).

1.1.5. Install the Exhaust Condenser on the Headplate

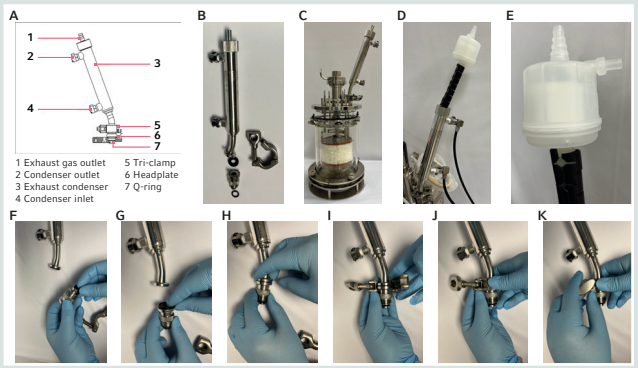

Figure 5: Install the exhaust condenser on the headplate. **(A)** Schematics. **(B)** Disassembled parts. **(C)** Fully assembled on the headplate. **(D)** The exhaust condenser in use, with water line connections and a 0.22 µm filter at the end. **(E)** Ensure the correct orientation of the exhaust filter during assembly. **(F-K)** See text section 1.1.5 for details.

> See Figure 5
> Before installation, lubricate the O-ring for the exhaust condenser and place it on the threaded portion of the sanitary fitting (Fig. 5F).
> Insert threaded portion of the assembled exhaust condenser into a Pg13.5 port on the headplate and secure by hand tightening.
> Note: The threaded portion of the sanitary fitting must be connected to the Pg13.5 port on the bioreactor headplate for the exhaust condenser installation before the tri-clamp is attached. However, for better visibility the headplate is not connected in Fig. 5F-K.
> Next, place the sanitary gasket on the bottom half of the sanitary fitting (Fig. 5G) and attach the exhaust condenser *via* tri-clamp connection (Fig. 5H-K). Make sure the condenser inlet and condenser outlet are facing away from the bearing housing to make it easier for water connections at the BioFlo 320 bioprocess control station.
> Attach a 1 - 2 inch (2.5-5 cm) piece of silicone tubing to the exhaust gas outlet with a 0.2 µm filter to create a sterile boundary. Secure with a cable tie (Fig. 5E).

1.1.6 Insert any headplate adapters, fittings, or plugs to complete the headplate

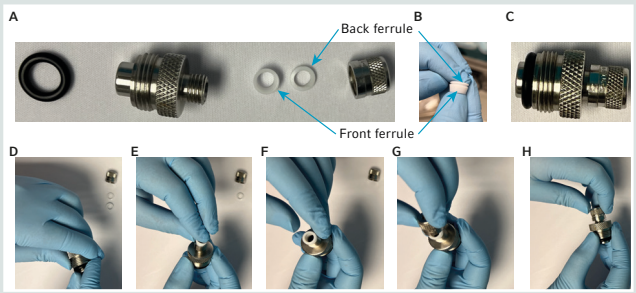

Figure 6: Assembly of a compression fitting. **(A)** Disassembled parts. **(B)** The orientation of the front ferrule and back ferrule. **(C)** The final assembled fitting. **(D-H)**. Steps demonstrating the assembly of a compression fitting.

> See Figure 6
> Check the O-rings for each port on the top of the headplate. O-rings are provided for all ports on the top of the headplate.
> Note: It is recommended that all O-rings are removed and cleaned after use. It is recommended that all O-rings should be replaced at least once a year or when they show wear. More frequent replacement of O-rings may be required depending on usage. Insert new O-rings after they have been lubricated with silicone grease.
> Check the front and back ferrules for each compression fitting. Fig. 6 shows the proper orientation and assembly of the ferrules in a compression fitting.
> Note: the teflon ferrules must be intact for proper function of a compression fitting. They are easily damaged or distorted during tightening.
> Assemble each adapter and fitting prior to installation. Fig. 7 shows the proper orientation and assembly of the individual pieces.
> Insert threaded portion of the adapters, fittings, addition and sample tubes and the thermowell. Make sure all connections are hand tightened.

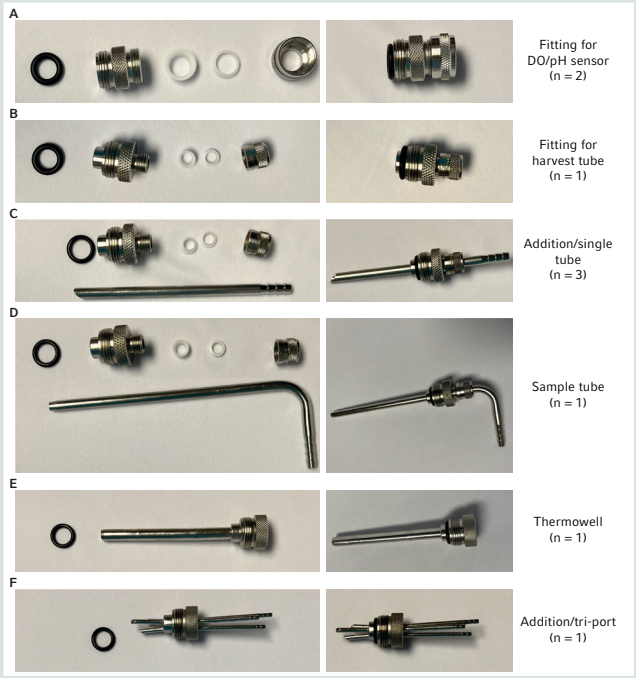

Figure 7: Headplate adapters and fittings: Left side shows the dissembled parts, right side shows the full assembly. **(A)** Compression fitting for DO/pH sensor. **(B)** Fitting for the harvest tube. **(C)** Addition (single) dip tube. **(D)** Sample tube. **(E)** Thermowell. **(F)** Tri-port. The O-rings contact the headplate and create the sterile boundary.

1.1.7. Insert the dissolved oxygen (DO) sensor in the headplate

> For DO sensors, maintenance varies by sensor type. Polarographic DO sensors contain electrolyte that must be changed based on usage. We recommend changing DO electrolyte before every run. Allow for polarization prior to use and after autoclaving. While changing the electrolyte, inspect the DO sensor membrane for wear. Replace if necessary.

> Gently insert the DO sensor through a Pg13.5 compression fitting on the headplate. Secure the sensor in place by turning the Pg13.5 compression fitting until the sensor is positioned at the desired depth in the vessel. The compression fitting must be tightened enough so that the sensor cannot move in the fitting.

> Place the sensor cap onto the DO sensor (included with the sensor). Autoclaving without the sensor cap may result in damage to the DO sensor electronics and break the sensor.

> Note: The DO sensor is calibrated after the vessel has been autoclaved and polarized.

1.1.8. Calibrate the pH sensor

> Connect the pH sensor to the appropriate pH cable first and then to the BioFlo 320 bioprocess control station. Twisting the cable may result in damage to the cable and may render it useless. Make sure that the sensor shows up in the set-up screen and the set-up screen is saved by pressing the check button.

> For cell culture conditions, we use our known pH 7.00 buffer and pH 10.00 buffer for calibration. It is recommended to condition the buffers at the same temperature as the working temperature employed for the cell culture run. Use approximately 25 mL of each buffer in a 50 mL tube.

> Calibrate the ZERO with pH 7.00 buffer. Select the calibration screen on the touchscreen of the BioFlo 320 bioprocess control station and select the pH sensor. Immerse the sensor in the pH 7.00 buffer. Wait until the pH reading stabilizes and press “set zero”. Type the 7.00 value into the keypad. Press the check button. The value has been saved. Remove and clean the electrode with DI water.

> Calibrate the SPAN with pH 10.00 buffer. Immerse the sensor in the pH 10.00 buffer. Wait until the pH reading stabilizes and press "set span". Type the 10.00 value in the keypad. Press the check button. The value has been saved. Remove and clean the electrode with DI water. The sensor is calibrated.

1.1.9. Insert the pH sensor into the compression fitting on the Headplate

> Gently Insert the pH sensor into the pH compression fitting on the headplate being careful not to break the sensor.

> Insert the pH sensor to its required immersion depth, which is usually just above the packed-bed impeller screen. Secure the sensor in place by tightening the compression fitting onto the pH sensor until the sensor will not move.

> Attach the sensor cap onto the pH sensor. Autoclaving without the sensor cap may result in damage to the pH sensor and break the sensor.

1.2. Preparing connections

1.2.1. Tubing

In a bioreactor run, it is often necessary to connect bottles in a sterile fashion. For such connections, we use a Terumo SCD® IIB Sterile Tubing Welder. This method allows the user to create a sterile connection to the bioreactor by fusing two pieces of sterile weldable tubing together through means of a tube welder. This tube welder can do both wet and dry welds. Other ways for sterile connections are described elsewhere (see Eppendorf Short Protocol No. 33). To prepare for the Terumo SCD® IIB Sterile Tubing Welder, all bottle connections for the bioreactor must have a weldable section of C-flex®. Additionally, we prepare extra pieces of C-flex tubing for tube welding needs. For prolonged cell culture processes such as a perfusion runs, we utilize a piece of Masterflex® tubing to use in the pump head, since it can better endure the mechanical stress from the pump head compression than silicone tubing over long periods of time.

The tubing is connected to each other by various sized connectors or reducers depending on the internal diameter (ID) of the tubing used. For enhanced sterility, the connection is secured with cable ties on both ends.

Note: To prevent C-flex tubing from rupture during autoclaving, one end of the tubing must be vented with a 0.22 µm filter.

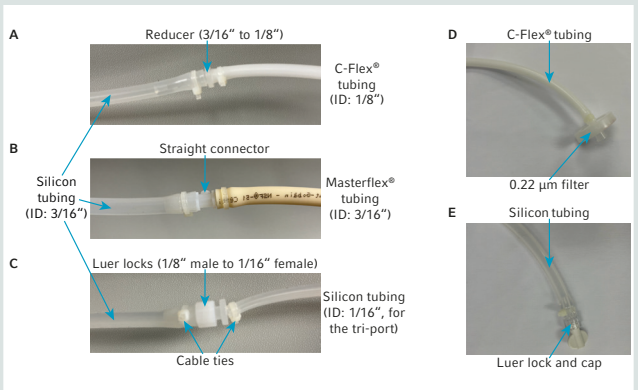

Figure 8: Tubing used for bottle connections to the bioreactor. **(A)** Silicone tubing connected to C-flex tubing via reducer to use with the tube welder. **(B)** Silicone tubing connected to Masterflex tubing via straight connector to use in the pump head. **(C)** Silicone tubing reduced to smaller silicone tubing via Luer lock connection to use on the tri-port connection. **(D)** End of C-flex tubing vented with a 0.22 µm filter. **(E)** End of silicone tubing capped with luer lock.

1.2.2. Tubing connections

Table 2: Tube connections.

No.	Part	Function	Connection
1	Harvest line	To transfer PBS or medium from the vessel	To a harvest bottle via a pump head on the controller
2	Media addition line	To add fresh medium into the vessel	To a media addition bottle via a pump head on the controller
3	Inoculum/ addition line	To add starting volume of media to vessel and/or inoculum into the vessel	To a bottle containing medium or inoculum
4	Sample line	To take samples from the culture	To sterile needleless syringe via needleless luer lock
5	Base line	For addition of sodium bicarbonate for pH adjustment	To a sterile bottle of 0.45M Sodium bicarbonate solution
6	Antifoam line	For addition of antifoam to suppress bubble formation	To a sterile bottle of 1 % antifoam C emulsion
7	Glucose bolus feed line	For addition of concentrated glucose bolus feed	To a sterilely prepared bottle of 200 g/L glucose solution
8	Sparge line	For sparging gases into the bioreactor	To a 0.22 µm filter
9	Blind port/ plugs	To fill any unused ports on the headplate	N/A

> For tubing connections of 1, 2, and 3, make a tubing extension consisting of a silicone tubing (ID 3/16", length ~20") that is connected to a C-flex tubing (ID 1/8", length ~10") via a reducer (1/8" to 1/32"). Attach the open end of the silicone tubing to the corresponding port, and the other open end to a 0.22 µm filter. Secure all connections with cable tie.

> For tubing connection of 4, attach an open end of a silicone tubing (ID 3/16", length ~4") to the sample port, and the other end to a Luer lock with needleless Luer lock and a Luer cap.

> For tubing connections of 5, 6, and 7, all bottles are connected via a tri-port. Since the tri-port has a smaller diameter tubing, the tubing extension would start from a silicone tubing of ID 1/16" that is connected to the same tube extension as described for tubing connections 1, 2, and 3. To make the connection, we use Luer lock connectors with corresponding male/female ends.

> For tubing connection of 8, attach a silicone tubing (ID 3/16", length ~10") to the upper sparge inlet located on the magnetic bearing housing and attach a 0.22 µm filter to the end. Secure all connection with cable ties.
> Fig. 9 depicts a possible configuration for the different tubing connections to the headplate (Fig. 9A) as well as additional details to the sparge line construction (Fig. 9B-D).
> Plug the remaining ports in the headplate, which are not in use, with stainless steel blind plugs. Secure the blind plugs in place by hand tightening.

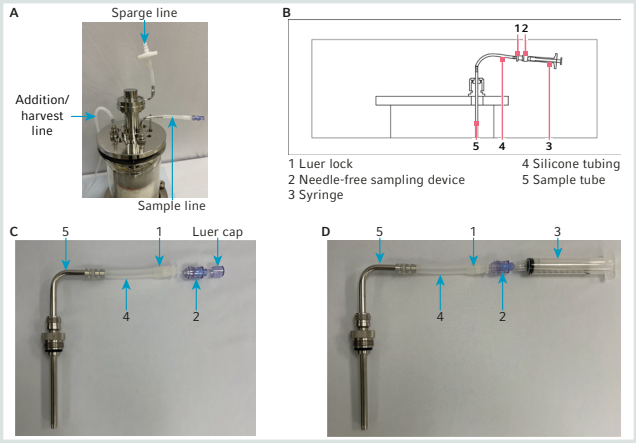

Figure 9: Tubing connections on the headplate. **(A)** Examples showing the liquid addition or harvest line, the sparge line and the sample line. **(B)** The sample line schematics. **(C)** The sample line parts. **(D)** Assembled sample line with syringe connection.

2. Pressure Hold Test

We recommend performing a pressure hold test to make sure that there are no leaks in any of the connections on the vessel headplate that could compromise sterility. The unit must be able to hold pressure at 3 psig (~0.2 bar), i.e. not exhibit a pressure drop greater than 0.2 psig (~0.013 bar) per hour at room temperature.

3. Autoclave the assembled glass bioreactor

3.1. Fill the vessel and the vessel jacket with liquid

> Fill glass vessel with Phosphate Buffered Saline (PBS). Fill the glass vessel with PBS through one of the larger ports on the headplate. The use of a funnel may be helpful. Alternatively, one of the tubing connections can be welded to a PBS bottle and used to pump PBS into the vessel. It is recommended to have the liquid level above the impeller blades for sterilization of the impeller interior region.
> Fill the vessel jacket with water. Connect the water inlet to the water outlet line on the vessel jacket to make filling easier. Allow the jacket to fill halfway. Do not fill the water jacket more than halfway. This could result in excess pressure build up in the jacket and cause a risk of vessel failure.

3.2. Final check of the assembled bioreactor before autoclaving

3.3. Autoclave the assembled bioreactor

Before autoclaving, follow the steps in the "Vessel Checklist prior to autoclaving" in Table 3. Autoclave the vessel on a minimum 30 minute liquid cycle. After autoclaving, the vessel will take time to cool down to room temperature (~ 2 hours). Allow the vessel to cool before making connections to the BioFlo 320 bioprocess control station.

Table 3: Vessel Checklist prior to autoclaving

No.	Specific items
1	All immersed lines (under liquid level) in the vessel are clamped
2	All silicone tubing is secured with cable ties
3	All filters are wrapped loosely in aluminum foil to prevent moisture entering the filters
4	All headplate ports not in use are closed by blind plugs
5	pH sensor is secured with sensor cap
6	DO sensor is secured with sensor cap
7	Bearing housing is covered with autoclavable cap
8	All immersed lines in the vessel are clamped. Note: It is critical to make sure that at least one venting line (not immersed) is open (exhaust).

4. Connect the bioreactor to a BioFlo 320 bioprocess control station

Figure 10: Completed vessel assembly ready for autoclaving.

4.1. Ground the bioreactor

The bioreactor must be properly grounded to the BioFlo 320 bioprocess control station before use. When you set up bioreactor and control station, the earth/grounding strap must be clipped to the bioreactor headplate to earth/ground the motor to the cabinet (Fig. 11A/B). Connect the loop end of the earth/grounding strap to the earth/grounding stud located on the back panel of the BioFlo 320 bioprocess control station, and screw on the hex nut to hold the strap in place (Fig. 11A/C). This is a requirement to meet safety regulations. Failure to ground the bioreactor to the BioFlo 320 bioprocess control station can result in electric shock and damage of the equipment.

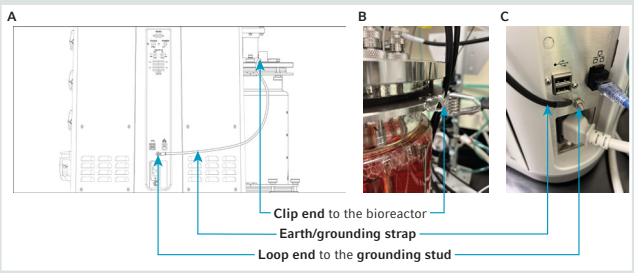

Figure 11: Grounding the vessel. **(A)** Schematics of the earth/grounding strap connecting the BioFlo 320 bioprocess control station on one end and the vessel on the other end. **(B)** The clip end of the earth/grounding strap to the upper retention ring of the vessel. **(C)** The loop end of the earth/grounding strap to the grounding stud secured by a hex nut.

4.2. Sensor, motor, temperature and sparge line connections

Connect each component of the bioreactor to corresponding ports on the BioFlo 320 bioprocess control station. Check that the configuration is correctly detected by the controller and save the configuration.

4.3. Water line connections

The water inlet and drain connections are located on the utility panel of the BioFlo 320 bioprocess control station (Fig. 12B). Quick-connect fittings are supplied with the control station for connection of utility hoses. At any time, be sure to follow these instructions in the order indicated. Incorrect connections or disconnections may lead to hose leakage and/or pressure build-up inside the vessel jacket.

> See Figure 12

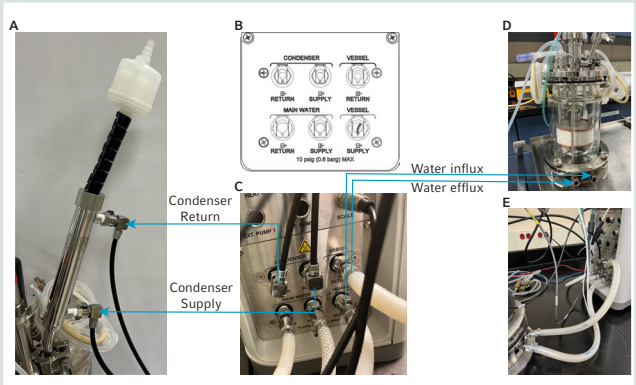

Figure 12: Water line connections. **(A)** Water line connection for the exhaust condenser. **(B)** Schematics of the utility panel of a BioFlo 320 control station. **(C)** Example water line connections. **(D)** The water jacket of a fully assembled packed-bed vessel. The inlet marked "Out" is connected to a tube inside the jacket. The inlet marked "In" is connected to the bottom of the jacket. **(E)** Example water line connections to the water jacket of a packed-bed vessel.

> Vessel jacket. Connect the quick connect hoses to the bioreactor jacket inlet and outlet (Fig. 12D). Connect the outlet marked “Out” on the vessel jacket to the “Return” quick connect located under “Vessel” of the BioFlo 320 control station utility panel (Fig. 12B/C). Connect the inlet marked “In” on the vessel jacket to the “Supply” quick connect located under “Vessel” of the BioFlo 320 control station utility panel (Fig. 12B/C). The outlet marked “Out” on the vessel jacket is connected to a long vertical tube inside to allow air to escape as water fills up the jacket. The inlet marked “In” on the vessel jacket is connected at the bottom of the vessel jacket.
> Main water. Connect the quick connect hoses to the “Supply” and “Return” under “Main Water” of the control station utility panel (Fig. 12B/C). The “Return” line should be connected first, so that there would not be a buildup of pressure inside the water drawer. This hose has a quick connect on one end and an open hose at the other end. The open end of the hose should be placed in a drain. The “Supply” line should be connected from the main water/house water outlet established in the facility to the quick connect on the control station utility panel. Water pressure from the main water/house water outlet must not exceed 10 psig (~0.69 bar) to the inlet on the BioFlo 320 bioprocess control station.
> Condenser. Connect the “Return” line on the exhaust condenser first via the condenser quick connect hose to the BioFlo 320 control station. Then, connect the “Supply” line on the exhaust condenser via the quick connect hose to the BioFlo 320 (Fig. 12A-C). Note that the “Supply” line is located at the bottom of the exhaust condenser and the “Return” line is located at the top of the condenser. Connect the lines accordingly to the exhaust condenser (Fig. 12A).

4.4. Pump calibration

Prepare a water-filled beaker and a graduated cylinder connected with a silicone tubing. The silicone tubing should be the same ID and length intended to use in the bioreactor run. Submerge one of the open ends of the tubing into the water filled beaker in an appropriate amount of water. Lay the other end of the tubing in a graduated cylinder. To calibrate pumps:

> Enter the gauge screen for the desired pump. Prime the line until the water has reached the end of the tubing. Press "Start" calibration on the calibration screen. The pump will automatically run for 3 minutes.
> Use the pop-up keypad to enter the amount of liquid that has been collected in the graduated cylinder.
> Note: Each pump can be calibrated individually, or the calibration can be applied to all of the same size pumps if the same tubing is being used.

4.5. Bottle connections

> Refer to Table 2 to make tubing connections to respective addition and harvest bottles. Clamp off all tubing connections.
> Assign pumps to respective bottles. Essential bottles include base, media addition and harvest.
> Note: The same type of pumps need to be assigned for media addition and harvest bottles for a perfusion run to maintain a constant working volume.

5. Inoculation

5.1. Medium conditioning

Since the vessel is filled with PBS after autoclaving, it needs to be removed and replaced with cell culture medium. The harvest line (Table 2) can be used for removing all the PBS from the vessel as well as adding in the starting volume of media. The medium needs to be conditioned prior to inoculation by turning on the temperature loop. The pH loop should be turned on prior to inoculation to ensure that the pH is close to the starting inoculum.

5.2. DO sensor calibration

Both optical and polarographic DO sensors can be used with the BioFlo 320. For polarographic DO sensors, the sensor needs to be immersed in the culture medium for >6 hours for polarization. Hereafter, the DO sensor can be calibrated. To calibrate a polarographic Intelligent Sensor Management (ISM®, Mettler-Toledo) DO sensor, only pressing "Set Span" is required.

> Turn on air at 0.5-1 SLPM for 30 minutes and with agitation, when the medium is fully saturated with dissolved O_2.
> Wait until the DO reading stabilizes and press the “Set Span” button. Type the value 100 in the pop-up keypad and press the check button to confirm the value.
> Change the air set point to a minimum flow of 0.005 SLPM.

5.3. Inoculum preparation

The cell number needed to inoculate a packed-bed of Fibra-Cel Disks is calculated based on an initial cell seeding density, total surface area of Fibra-Cel Disks, and the total weight of the Fibra-Cel Disks. The inoculum is typically prepared as a concentrated cell suspension.

5.4. Inoculation

The inoculum bottle is connected to the bioreactor via tube welding. To introduce the inoculum into the bioreactor, the liquid flow needs to follow the design principles of the packed-bed impeller. The impeller consists of a hollow body with an open end at the bottom that is connected to the exit openings of the horizontal impeller arm.

> The horizontal impeller arm has an asymmetric exit opening. When it rotates clockwise with the longer part of the horizontal impeller arm acting as the leading edge, a pressure differential is generated to the shorter part of the impeller arm. This creates a suction force at the exit opening, pulling liquid upward through the hollow body of the impeller and out of the exit opening. Outside the impeller, the liquid would move downward and pass through the packed-bed. The flow dynamics are illustrated in Fig. 13.

> For adherent cell culture, it is critical to shorten the duration of cells in suspension during the initial seeding. Therefore, it is important to have the cells make contact to the Fibra-Cel Disks as quickly as possible during the inoculation process. It is also necessary to have even cell distribution throughout the packed-bed. Based on the flow path in the packed-bed bioreactor, cells need to be seeded below the packed-bed impeller, e.g. the harvest line so that the cells are evenly distributed throughout the packed-bed.

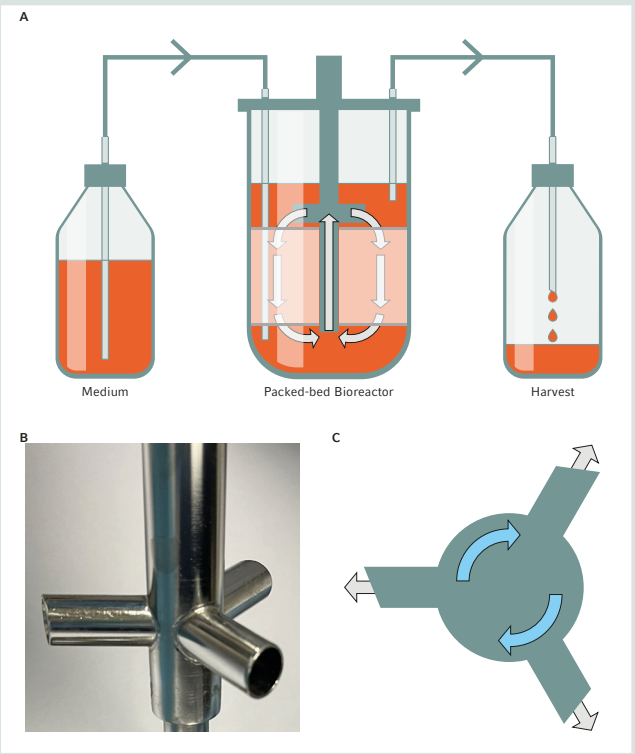

Figure 13: (A) Schematics of the medium flow path through the impeller of a packed-bed bioreactor. **(B)** Close-up of the horizontal impeller arms with asymmetric exit openings. **(C)** Schematic top-down view of the horizontal impeller arms. Clockwise rotation (blue arrows) creates a pressure differential between the longer and shorter edge of the impeller arm which results in a suction force at the exit opening, pulling liquid upward through the hollow body assembly of the impeller and out of the exit opening (grey arrows).

6. Perfusion

6.1. Perfusion setup

6.1.1. Medium "In" and perfusate "Out" bottles.

Two separate bottles are prepared; a fresh medium addition (Medium "In") and a spent medium harvest (Perfusate "Out"). They are then connected to the bioreactor (see Table 2). It is highly recommended to have both bottles on separate scales independent of the controller, to accurately quantify medium exchange throughout the perfusion run.

Note: Because of the need for large volume of medium exchange for a perfusion run, it is often necessary to swap the medium "In" and/or empty the perfusate "Out" bottle. Additionally, it may be necessary to take samples from the perfusate "Out" bottle. Therefore, it is helpful to have additional lines connected to the reservoir bottles with a Y-connector. This allows additional bottles to be welded on or off for sterile medium exchange without having to maneuver the large reservoir bottles.

6.1.2. Volume loop setup

To maintain a fixed working volume in the bioreactor, the "In" and "Out" medium exchanges need to match. One way to do this is to set the same time profiles for both pumps. However, even small discrepancies between the pumps may lead to overfeeding or underfeeding the bioreactor. Alternatively, a volume loop can be set up such that the setpoint (SP) of the volume is a constant and pumps are only activated when the present value (PV) of the volume deviates from the SP.

> Volume setpoint. Fill the bioreactor with culture medium at the desired level. Mark the present value of the volume as the volume SP.
> Assign "Medium Addition" and "Harvest" pumps. Place the tubing lines for the medium "In" and "Out" bottles to their respective pumps, and assign the pumps accordingly. Make sure that the direction of the pump head rotation is correct, based on which way the medium needs to flow (either into the vessel or out of it).
> Set "Time Profile" of the "Medium Addition" line. The medium can be added into the vessel at a fixed flow rate for perfusion, or with a feeding schedule using the "Time Profile" function on the BioFlo 320.
> Set "Flow Rate" of the "Harvest" line. The flow rate for the "Harvest" line needs to be higher than the highest rate of the "Medium Addition" to prevent failure of the volume loop.

6.1.3. Feeding and perfusion control

The feeding strategy in all perfusion runs aims at keeping the concentration of growth-inhibiting compunds like lactate and ammonium below toxic levels. In addition, glucose bolus may be needed to maintain a concentration between 1-4 g/L. Therefore, the metabolite levels are measured daily, and the perfusion rate adjusted accordingly to maintain these target levels. Both ammonium and glucose can be measured daily, e.g. with a CEDEX® Bio Analyzer (Roche Diagnostics®).

6.1.4. Monitoring glucose consumption

Because the cells adhered to the Fibra-Cel Disks, the cell density cannot be directly measured during the bioreactor run. As an indirect measure of cell growth, the glucose consumption rate of the culture is tracked. The total glucose consumption of the culture and the daily glucose consumption rate can be calculated based on the formula previously described in our application note 359 [3].

6.2. Terminating cultures – crystal violet nucleus counting assay

The final yield of cell numbers in the packed-bed bioreactor can be measured by a crystal violet nucleus counting assay (Chemglass Life Science, USA, CLS-1332-01). After the completion of the cell culture process, the bioreactor is disassembled and the packed-bed screen top is removed out of the vessel. Samples of Fibra-Cel Disks containing the same number of Fibra-Cel Disks are transferred to 50 mL conical tubes. Cell nuclei from the Fibra-Cel Disks are extracted and stained according to instructions supplied with the crystal violet dye nucleus count kit. The cell nuclei can be counted using a VI-CELL® Cell Viability Analyzer (Beckman Coulter®, USA) with the "default" cell type setting as described in our application note 359 [3].

6.3. Extrapolate cell growth curve from glucose consumption rate curve

Cell densities throughout the bioreactor run can be extrapolated from the glucose consumption rate of the culture. First, the glucose consumption to cell density conversion ratio is calculated from the final glucose consumption rate and cell density measured with the crystal violet nucleus counting assay. Assuming a constant conversion ratio over the duration of the culture, the daily glucose consumption rate is converted into a daily cell density. For more information, see our application note 359 [3].

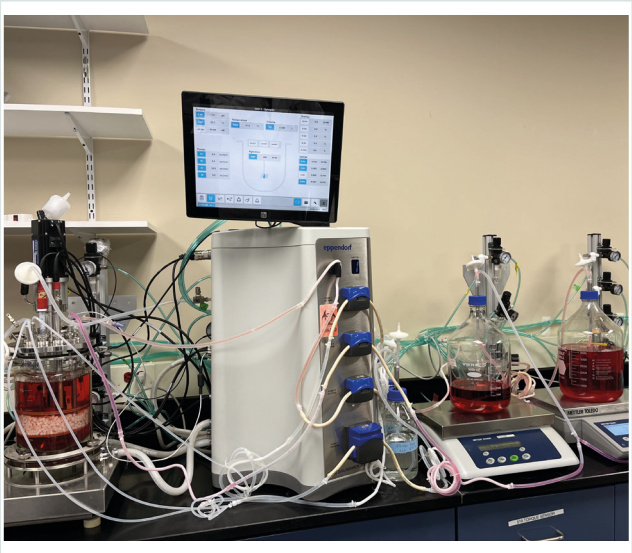

Figure 14: Asssembled packed-bed Fibra-Cel Disk bioreactor connected to a BioFlo 320 bioprocess control station for perfusion culture.

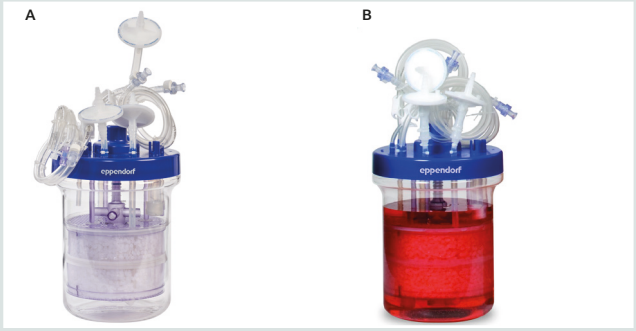

Figure 15: **BioBLU® 5p Single-Use Bioreactor.** This single-use packed-bed bioreactor alternative to our glass bioreactors comes ready-to-use and pre-loaded with Fibra-Cel Disks. Compared to glass vessels, it offers reduced cleaning effort, faster turnaround times, and is perfectly suited for adherent cells and perfusion culture. **(A)** As delivered and **(B)** Filled with medium ready for adherent cell culture runs.

Learn more about of the Eppendorf single-use solutions for your approach at: Eppendorf Single-Use Bioreactors

Examples

Here is a summary of the previously published application notes on the use of packed-bed Fibra-Cel bioreactors.

Example 1: Application note 336 [2] - Perfusion CHO Cell Culture in a BioBLU® 5p Single-Use Bioreactor (packed-bed) (from earlier studies in the Eppendorf labs)

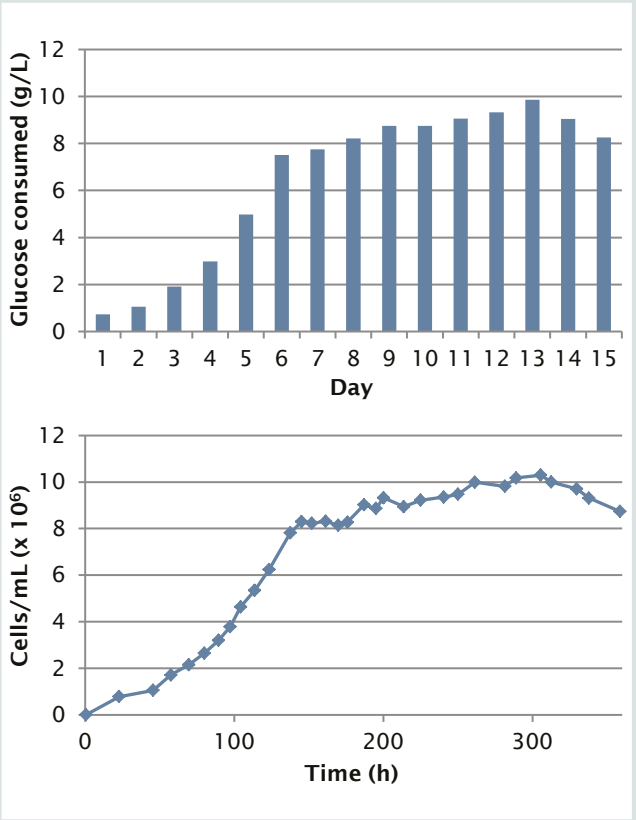

Figure 16: Perfusion CHO cell culture in a BioBLU 5p Single-Use Bioreactor (packed-bed). **(Top)** Glucose consumption rate curve. **(Bottom)** Cell density curve.

Example 2: Application note 359 [3] - High-density Vero cell perfusion culture in BioBLU® 5p Single-Use Bioreactor (packed-bed)

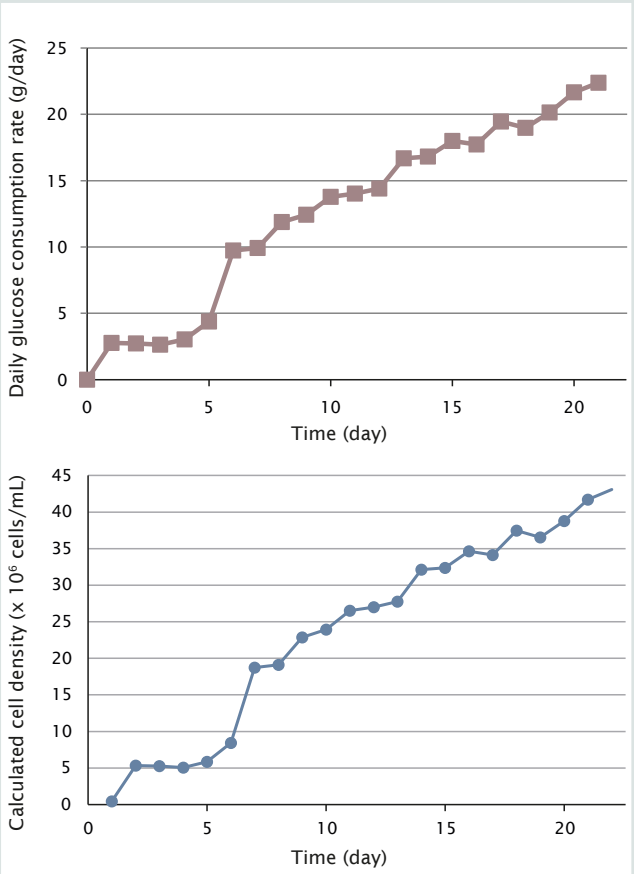

Figure 17: High-density Vero cell perfusion culture in BioBLU 5p Single-Use Bioreactor (packed-bed). **(Top)** Glucose consumption rate curve. **(Bottom)** Calculated Vero cell growth curve.

Example 3: Application note 258 [4] - Hybridoma culture using New Brunswick CelliGen® 310 with packed-bed Fibra-Cel Disk impeller

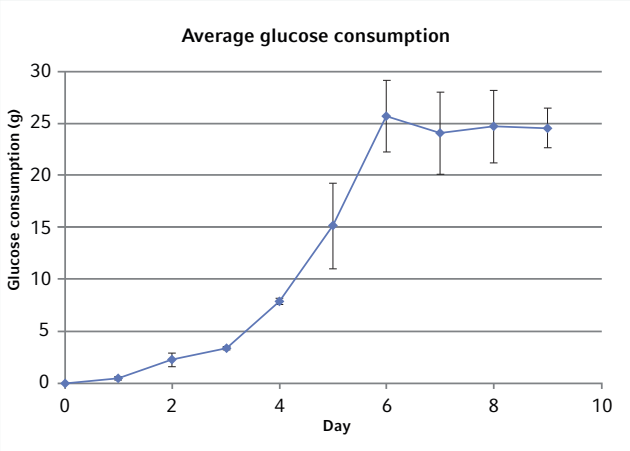

Figure 17: Hybridoma culture using New Brunswick CelliGen 310 with packed-bed Fibra-Cel Disk impeller. Glucose consumption rate curve.

Example 4: Application note 411 [5] - Transient Lentiviral vector production in HEK 293T cells using the BioFlo 320 control station with a BioBLU 5p Single-Use Bioreactor (packed-bed).

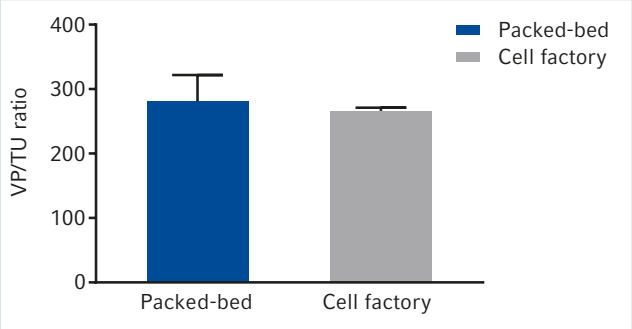

Figure 18: Transient Lentiviral vector production in HEK 293T cells using the BioFlo 320 control station with a BioBLU 5p Single-Use Bioreactor (packed-bed). The VP (vector particles) to TU (functional unconcentrated titers) ratio demonstrates comparable vector preparation quality between packed-bed and cell factory production methods.

Literature

[1] Mirro R. An Update on the Advantages of Fibra-Cel® Disks for Cell Culture What Is Fibra-Cel? Eppendorf Application Note 313; https://www.eppendorf.com/product-media/doc/en/70429/New-Brunswick_Fermentors-Bioreactors_Application-Note_313_Fibra-Cel_An-Update-Advantages-Fibra-Cel-Disks-Cell-Culture.pdf

[2] Kohlstrom N, Sha M. Perfusion CHO Cell Culture in a BioBLU® 5p Single-Use Packed-Bed Vessel. Eppendorf Application Note 336

[3] Han X, Sha M. High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels. Eppendorf Application Note 359; https://www.eppendorf.com/product-media/doc/en/308208/Fermentors-Bioreactors_Application-Note_359_BioBLU-5p_High-Density-Vero-Cell-Perfusion-Culture-BioBLU-5p-Single-Vessels.pdf

[4] Rose R, Willard S, Sha M. Hybridoma culture using New Brunswick TM CelliGen® 310 with Packed-bed Fibra-Cel® Basket Impeller. Eppendorf Application Note 258; https://www.eppendorf.com/product-media/doc/en/68531/New-Brunswick_Fermentors-Bioreactors_Application-Note_258_CelliGen-310_Hybridoma-culture-Eppendorf-New-Brunswick-CelliGen-310-Packed-bed-Fibra-Cel-Basket-Impelle.pdf

[5] McCarron A, Donnelley M, McIntyre C, Parsons D. Transient Lentiviral Vector Production in HEK 293T Cells Using the BioFlo® 320 Control Station with a BioBLU® 5p Single-Use Packed-Bed Vessel. Eppendorf Application Note 411; https://www.eppendorf.com/product-media/doc/en/782013/Fermentors-Bioreactors_Application-Note_411_BioFlo-320_Transient-Lentiviral-Vector-Production-HEK-293T-Cells-BioFlo-320-Control-StationBioBLU-5p-Single-Packed-Bed-Vessel.pdf

Ordering information

Description	Order no.
BioFlo® 320, base control station, with water connection	1379 963 011
BioFlo® 320 sparge gas option, 4 TMFC (0.002 – 1.0 SLPM)	1379 501 411
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 1 L	M1379-0300
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 3 L	M1379-0301
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 5 L	M1379-0302
Vessel Bundle, for BioFlo® 320, stainless-steel dished bottom, direct drive, 10 L	M1379-0303
Single-Use Bioreactor Bundle, for BioFlo® 320, for BioBLU® 5p	M1379-0323
Packed-Bed Impeller Kit, for BioFlo® 320, for 1 L vessel	M1379-1140
Packed-Bed Impeller Kit, for BioFlo® 320, for 3 L vessel	M1379-1141
Packed-Bed Impeller Kit, for BioFlo® 320, for 5 L vessel	M1379-1142
Packed-Bed Impeller Kit, for BioFlo® 320, for 10 L vessel	M1379-1143
Fibra-Cel® Disk, 50 g	M1292-9984
Fibra-Cel® Disk, 150 g	M1292-9992
Fibra-Cel® Disk, 250 g	M1292-9988
Fibra-Cel® Disk, 1 kg	M1292-9974
BioBLU® 5p Single-Use Bioreactor, microsparger, packed-bed impeller, optical pH, sterile, 1 piece	M1363-0119
BioBLU® 5p Single-Use Bioreactor, macrosparger, packed-bed impeller, optical pH, sterile, 1 piece	M1363-0133

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com/bioprocess