

## ☰ Protocol

# Performing a manual run on your Xuri™ Cell Expansion System W25

CANCER RESEARCH ★

ANALYTICAL TESTING

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## Abstract

The purpose of this protocol is to provide instructions on how to perform a manual run on the Xuri™ Cell Expansion System W25 using UNICORN™ software.

This protocol applies to anyone performing a manual run on the Xuri™ Cell Expansion System W25.

## Introduction

This protocol outlines how you can use UNICORN™ software to perform a manual run on your Xuri™ W25 system.

## Reagents and Equipment

### Equipment

Xuri™ Cell Expansion System W25 and components

Computer with UNICORN™ Software

Xuri™ Cellbag™ bioreactor

### Reagents

Xuri™ T Cell Expansion Medium

### Personal protective equipment

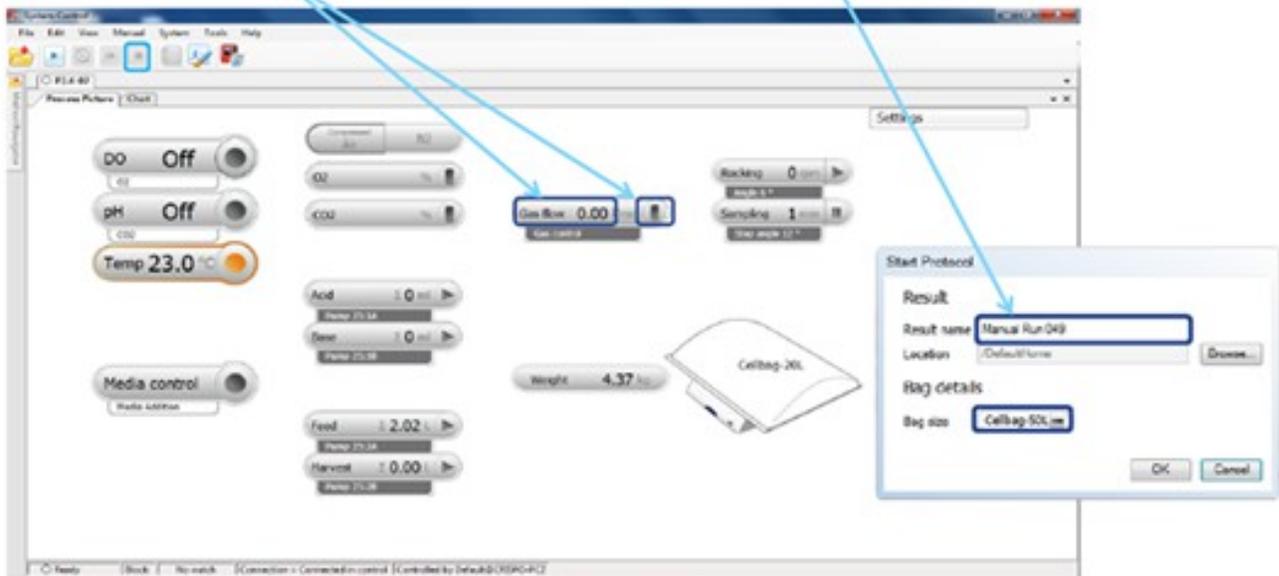
- Laboratory coat
- Safety goggles
- Gloves

## Procedure

# Start a manual run

1. Click left-hand side of button to edit and acknowledge settings or click right-hand side of button. Start Protocol appears

2. Enter result name and Cellbag™ size in Start Protocol to initiate and log the run.



Click "Stop" in toolbar to end the run

# Process picture features

The screenshot displays the UNICORN software interface with a process picture. The interface includes a top menu bar with options like 'System Control', 'Administration', 'Tools', 'Help', and 'About'. The main area shows a process picture with various control elements: DO (Off), pH (7.30), Temp (26.7), Media control (Media Address), Gas flow (0.21), Backing (25), Weight (4.20), Feed (0.10), Harvest (0.00), and a Cellbag 20L. A 'Settings' window is open on the right, showing 'Media control' settings. Callouts provide instructions: 'To access more advanced functions, click Manual: Execute manual instructions', 'To calibrate, click System: Calibrate and select monitor of choice', 'To edit system configuration, e.g. add pump/CBCU, click Tools and select Administration: System Properties: Edit', 'Click Connect icon to view or control another system', 'OK: Confirm the setting', 'Tab: Go to next input field', 'Enter: OK', 'Esc: Cancel', 'Point (.): Decimal divider', 'Click Cellbag icon to enter calibration values for pH and DO', 'Frame and button colors: Green: Function is active, Orange: Attention needed, click on the button to view more information, Red: Function is not working properly, click on the button to view more information', 'Lines indicates control hierarchy, e.g. Media control commands the feed pump', and 'For contextual help, click F1'.

To access more advanced functions, click **Manual: Execute manual instructions**

To calibrate, click **System: Calibrate** and select monitor of choice

To edit system configuration, e.g. add pump/CBCU, click **Tools** and select **Administration: System Properties: Edit**

Click **Connect** icon to view or control another system

OK: Confirm the setting  
Tab: Go to next input field  
Enter: OK  
Esc: Cancel  
Point (.): Decimal divider

Click **Cellbag** icon to enter calibration values for pH and DO

**Frame and button colors:**  
**Green:** Function is active  
**Orange:** Attention needed, click on the button to view more information  
**Red:** Function is not working properly, click on the button to view more information

Lines indicates control hierarchy, e.g. Media control commands the feed pump

For contextual help, click F1

Xuri Cell Expansion System W25 Cue Card 29-0878-22 AA

## 5.1 Connect System and Start Run

5.1.1 Turn on the Xuri™ W25 system using the button on the base of the machine. It will flash green until connected to the computer. Open the UNICORN™ software.

5.1.2 At the UNICORN™ log in screen, use either DEFAULT username (no password required) or appropriate user account. Four screens should appear (Administration, Systems Control, Method Editor, and Evaluation).

5.1.3 Under "System Control," use the red icon to connect to the Xuri™ W25 system as indicated by its serial number located on the front of the machine.

5.1.4 A Cellbag™ bioreactor should be attached to the tray with all appropriate connections made (filter heater to the outlet gas filter, tubing from GAS MIX OUT to the inlet gas filter, FEED and HARVEST tubing loaded into pump heads).

5.1.4a If DO/pH is being monitored, Cellbag™ optical sensors should be employed.

5.1.4b For perfusion cultivation, the FEED and HARVEST pumps should be calibrated to reach optimal precision either before run or using Auto-calibration during run.

5.1.5 Start a manual run by clicking on the right-hand side of any icon. The "Start Protocol" window will appear.

5.1.6 Under "Start Protocol," enter the desired run name and Cellbag™ size.

5.1.7 **If using DO/pH sensors introduce bag manufacturing calibration values in the appropriate fields** (values are on stickers on the upper surface of the cell bag)

## 5.2 Initiate run

5.2.1 To inflate the bag, ensure all ports on the Cellbag™ bioreactor are closed, and the inlet and outlet filters are open. Type in your gas flow and CO2 percentage of choice and click OK. Turn on "Gas Flow and CO2" on the process picture by clicking the right hand side of the button. Inflate the Cellbag™ bioreactor using the "Fast fill" function under "Gas control" à "Gas flow."

5.2.2 Once the Cellbag™ bioreactor is inflated, press "Disable." **Push down on the bag around the filters** to ensure the seals have broken.

5.2.3 To tare the system, open "Settings" > "Weight." Ensure the lid is placed on the tray and it is closed. Tray should be horizontal (stop angle 0). Click "Tare."

5.2.4 To add media, open "Rocking" on the process picture. Change the "Slope angle" to 12.0 and click "OK." Transfer the desired volume of medium into the Cellbag™ bioreactor using a pump or gravity flow. **To automatically fill the bag to a desired weight, use "Media Addition" from "Settings" > "Control," by typing in the required set point. Ensure the flow rate is set to an appropriate value. (L/day).**

5.2.4a When using the pump, press FEED button and change the RPM "Speed" to give a desirable "Flow rate" in (mL/minute).

5.2.4b Stop FEED by pressing green arrow key to grey when it reaches the desired volume

5.2.5 To equilibrate the culture media, set the desired rocking speed and angle in "Settings" > "Rocking." Start the rocking by clicking the right hand side of the "Rocking" button. Click on the right hand side of the "Temperature" button to start heating. **Run for a minimum of 2 hours to reach the desired operating conditions. If using DO/pH sensors these should be set to "read" at this stage.**

5.2.6 After equilibration calibrate DO and pH sensors if applicable.

5.2.6a DO sensor calibration: Select System: Calibrate in System Control. Select the DO sensor in the Monitor to calibrate drop down menu. Enter percentage of air saturation (95%) depending on CO2 concentration (normally 5%) in the Enter reference DO field. Click Calibrate. Close the Calibration dialog.

5.2.6b pH sensor calibration: Take a sample to verify that the pH value shown by the system matches the pH measured with a calibrated reference instrument. If the deviation is larger than approximately 0.5 pH units, make sure that no air bubble is present. Select System: Calibrate in System Control. Select the pH sensor in the Monitor to calibrate drop down menu. Enter the actual pH value in the Enter reference pH field. Click Calibrate. Close the Calibration dialog.

5.2.7 To inoculate cells, connect the tubing from the inoculum container to the inlet tubing by sterile welding.

5.2.8 Unclamp the inlet tubing and inoculum container tubing. Transfer the desired volume of inoculum into the bag using media control, direct pumping or gravity flow.

5.2.9 Wash the feed line with fresh culture media to ensure that all the cells reached the Cellbag™ bioreactor by sterile welding a media bag to the line.

### 5.3 During run

5.3.1 Real time monitoring of parameters is offered through the UNICORN™ software platform. This data can be viewed as curves in "Chart" tab.

5.3.1a User can customize these curves under "Tools" à "Customize" > "Curves."

5.3.2 Manual manipulation with the system can be performed using the "Process picture."

5.3.3 To sample, increase the rocking rate to at least 15 RPM and allow mixing to occur for a few minutes.

5.3.4 Click the right hand side of the "Sampling" button. The system will enter sampling mode. The system will stay in this mode for as long as the time is set under "Settings" > "Rocking" > "Sampling" > "Stop angle" in the "Process picture."

5.3.5 To enter perfusion mode, click "Settings" > "Media control" > "Media perfusion" and set the desired flow rate. Press "OK" and the "Media Control" button will turn green. Perform pump autocalibration at the beginning of the perfusion culture.

5.3.5a Autocalibration: Change the flow rate to **10L/day**. Select OK for auto-calibration. Auto-calibration will run immediately and takes approximately 30mins (this period should be timed as the machine will not signal the end of the autocalibration). After auto-calibration is complete change the flow rate to your desired perfusion rate/day minus 100ml.

## 5.4 End run

5.4.1 To end the cultivation and harvest, prepare the harvest vessel. Click the "Stop" button on the top toolbar in "System control." Click "OK."

5.4.2 Connect tubing from the Cellbag™ bioreactor to the harvest vessel. Empty Cellbag™ bioreactor using either a pump or gravity flow.

5.4.3 Clamp the inlet and outlet filters and other tubings. Disconnect all tubings and cables. Remove the empty Cellbag™ bioreactor and discard.

5.4.4 Turn off all gas supplies.

5.4.5 Disconnect from the system in UNICORN™ software and switch "OFF" system.

5.4.6 Clean system units with an alcohol impregnated wipe.

## 5.5 Additional features

5.5.1 To monitor DO/pH, open "Settings" > "Cellbag" > "Left/Single" and select the correct Cellbag™ size prior to the run. Enter the Bag sensor calibration data.

5.5.2 After media addition, click "Settings" > "DO" and set to "Reading On."

5.5.3 Click "Settings" > "Ph" and set to "Reading On."

5.5.4 Wait until values are stable (approximately 2 hours).

5.5.5 Perform the DO calibration by selecting "System" > "Calibrate" in "System control." Select "DO sensor" in the "Monitor to calibrate" menu. Enter the percentage of air saturation (95%) depending on the CO2 concentration (5%) in the "Enter reference DO" field. Click "Calibrate" and close window.

5.5.6 To perform the pH calibration, take a sample to verify the pH value with a calibrated reference instrument. Make sure no air bubble is present. Select "System" > "Calibrate" in "System control." Select "pH sensor" in the "Monitor to calibrate" menu. Enter the actual pH value in the "Enter reference pH field." Click "Calibrate" and close window.

5.5.7 To manually calibrate the pump, ensure the flow rate set-point is entered correctly. To give a 10 mL/minute calibration value, set a flow-rate of 14.4 L/day. Enter the appropriate inner tubing diameter in "Pump" tab (1.6 mm or 3.2 mm) and click "System" > "Calibrate." Select pump to calibrate, perform calibration, and record result obtained. Repeat until value is approximately 10 mL

5.5.7a to ensure accurate weight, use an open end tubing and lab bottle to verify weight before and after calibration.

5.5.8 To auto-calibrate the pump, ensure the perfusion rate is set **above 3.4 L/day**. Select "Media control" > "Perfusion" and change the flow rate to 10 L/day. Select "Auto calibration" "ON" and process will occur immediately. This process lasts for approximately 30 minutes. When complete, change the flow rate back to desired rate/day minus 100 mL. Turn "Auto-calibration" "OFF."

## Time Taken

30 minutes

## Notes and Comments

### 6.1 Additional information

6.1.1 UNICORN™ system control software consists of four modules: Administration, Method Editor, System Control, and Evaluation. A comprehensive help tool is also included.

6.1.1a **Administration:** The Administration module is used to administer all functions of the UNICORN™ software. Available functions include user and email setup, controlling access to groups and network users, defining and editing system properties, database management, and logging records of usage and activity.

6.1.1b **Method Editor:** Instructions to control a bioreactor run can be defined in a method. The Method Editor module is used to create, edit, save, and work with methods. An existing method can be changed to simplify the editing process. Individual changes can be saved for later use on systems having the same instrument and component configuration.

6.1.1c **System Control:** The System Control module is used to connect to the system as well as to start, view, and control a run. Default parameter values for an instrument can be viewed and edited in the System Settings dialog before a run is started or during an ongoing run using manual instructions. It is also possible to connect to other systems.

6.1.1d **Process Picture:** This panel enables manual interaction with the system and provides the status of the run parameters. Data is shown in the Process Picture but can also be viewed as curves in the Charts tab and as numerical values in the Run Data pane. Curves and information about the run are saved in a database, which can be opened in the Evaluation module. The default view shows the most commonly used curves. The user may customize which curves to display and the color and style of the displayed curves.

6.1.1e **Evaluation:** In the Evaluation module, content of the result files can be viewed, analyzed, and compiled as reports. Reports can be customized, saved, and printed as required

6.1.2 **Alarms, notifications, and data logging:** UNICORN™ software provides access to all operations, data, and alarm conditions. A dynamic graphical user interface shows the real-time status of the run while data are automatically saved. Alarms can be configured to alert the user when certain conditions are met. Individual deviation alarms can be set for all essential parameters (e.g., heating, gas flow, CO<sub>2</sub>, pH, and DO). Alarm sensitivity and delay is defined by the user. If an alarm condition is triggered, the source of the alarm is highlighted in the graphical user interface and information about the alarm, such as date and time of occurrence as well as a description of the cause and how to solve the problem are displayed in a dialog box. Alarms can also be configured to send email notifications of alarms and errors to an address of your choice.

### 6.2 Waste disposal

Waste arising from this process should be disposed of via the appropriate waste stream.

### Definitions and Abbreviations

CO<sub>2</sub> Carbon dioxide

DO Dissolved oxygen

THIS PROCEDURE IS TO BE PERFORMED BY TRAINED PERSONNEL ONLY.

