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ReadyToProcess WAVE 25

Method template user guide

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1 Introduction

ReadyToProcess WAVE 25 bioreactor system

The single-use ReadyToProcess WAVE™ 25 bioreactor system is a reliable and intuitive cell culture device for working volumes up to 25 L.

The system is based on a well-known rocking technology that provides mixing and aeration to the culture, which is maintained in an inflated, disposable Cellbag™ bioreactor cultivation chamber.

With ReadyToProcess WAVE 25, the rocking technology is enhanced with features such as advanced sensors and intelligent control strategies. System operation is easily managed via the intuitive and user friendly UNICORN™ system control software.

The system is designed for fast installation and convenient handling, and delivers a reliable and accurate performance suitable for research, process development, and manufacturing operations.

UNICORN software

UNICORN software provides full automation with data logging capabilities of the entire cultivation process. UNICORN can be used in a 21 CFR Part 11 compliant way, satisfying requirements for electronic records and signatures to meet increasing regulatory demands.

Template methods

ReadyToProcess WAVE 25 template methods offer a starting point for method creation. ***Templates*** are basic methods that can be used as a starting point for developing customized methods. The method variables in a suitable ***Template*** are adjusted to create a method for the current application.

2 Safety

ReadyToProcess WAVE 25 Operating Instructions describes how to use the systems safely. Read the safety instructions in this manual before installing, using, or maintaining the system.

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ReadyToProcess WAVE 25

Operating Instructions

Original instructions

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3 Template methods

In this chapter

3.1 Overview and import instructions

Available template methods

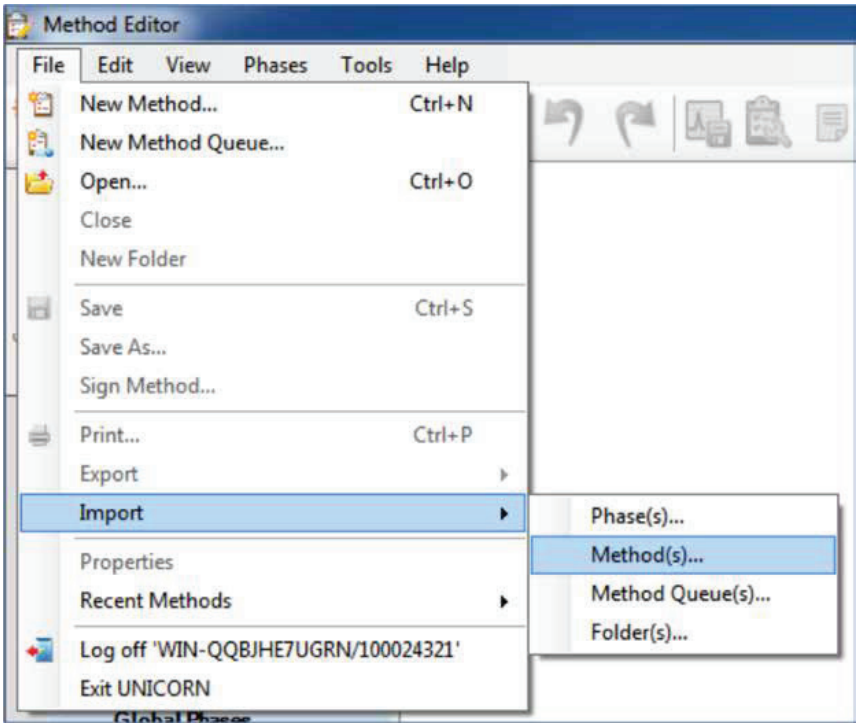
A set of templates is available for automatic control of the ReadyToProcess WAVE 25 system.

The templates cover

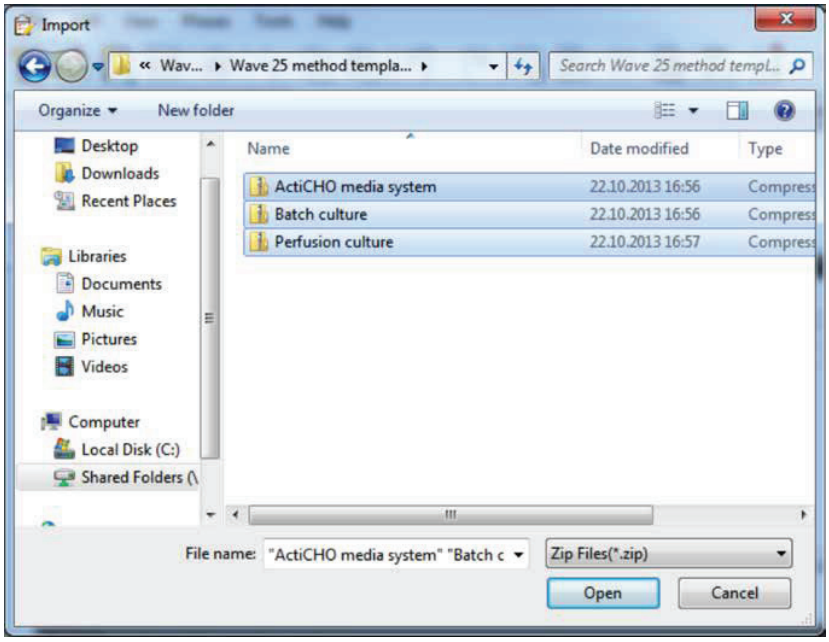
- Batch culture
- Perfusion culture
- Fed-batch culture using the ActiCHO™ Media System

Importing methods

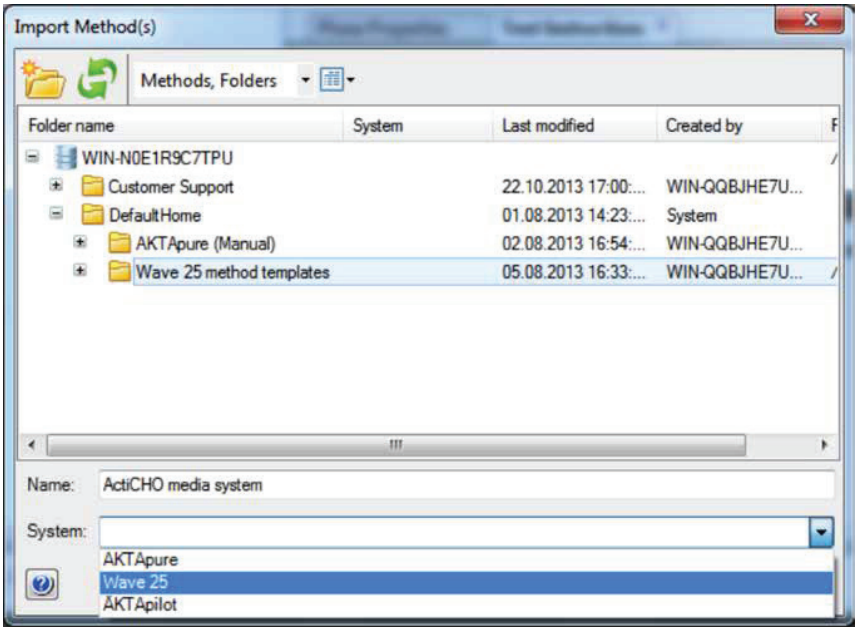
The methods are delivered in .zip format. To import them into UNICORN, open the ***Method Editor*** and select the menu item ***File > Import > Method(s)...***



Select the location in which you saved the .zip files, highlight the method(s) you wish to import, and click ***Open***.



In the next step, you have to select the location to which the methods should be imported in UNICORN and the system on which the methods will be run. Depending on how the installation of the system was done, the location might have a different name than WAVE 25.



Click on ***Import*** to import the method(s) into the selected folder.

3.2 Method details

In this section

3.2.1 Batch culture

ReadyToProcess WAVE 25 (1.2.0.1) or higher, UNICORN 6.3.2 or higher

Method for running a batch culture using ReadyToProcess WAVE 25 includes setup of the system, Cellbag bioreactor installation, inflation of the bioreactor bag, media fill, equilibration, probe calibration, inoculation, pH and dissolved oxygen (DO) control, and cultivation phase.

The method uses variables, which are part of an instruction (e.g., a parameter) and has default set points that can be changed during startup of the run in the ***Start protocol variables*** pane.

Note: *This is a template that should cover most batch cultivation requirements. The user needs to set parameters to set points suitable for the specific process during startup of the run in the* ***Start protocol variables*** *pane. Depending on the specific process, the choice given by the variables might not be enough and changes to the text instructions might be necessary.*

Phase ***Method Settings***:

Sets the Cellbag size (selected in ***Variables*** pane during startup) and gives notifications to attach probe cables, install the Cellbag bioreactor, and attach air line and filter heater.

Phase ***Cellbag Inflation***:

Sets the air source and gas flow settings (variable), starts fast fill for inflation, sets the CO2 mix parameters (variable), and notifies the user to connect base / acid bottle for pH control (if required) and to install the lid. Finally, the scale is tared.

Phase ***Media Fill***:

Notifies the user to attach the cell culture media vessel to the Cellbag bioreactor and install the pump tubing, and then starts the media fill up to the set point given in ***Variables***. Holds until the set point has been reached, after which a message informs the user to disconnect the medium vessel.

Phase ***Equilibration***:

Starts rocking at the speed and angle defined in ***Variables*** during startup. Starts heating (set point defined in ***Variables***). Duration 2 h.

Phase ***Probe Calibration***:

Notifies the user to perform DO and pH calibration, including pH offline measurement and calibration.

Phase ***Inoculation***:

Notifies the user to attach the inoculation vessel to the Cellbag bioreactor and to install the pump tubing. After this step, inoculation is started by running in media control mode up to the required total weight of the Cellbag bioreactor, after which a message informs the user to disconnect the inoculation vessel.

Phase ***Initiate pH and DO Control***:

Start of DO and pH control according to settings defined in ***Variables***.

Phase ***Batch Culture***:

Empty phase with duration of 5000 hours. At any point during this phase, manual adjustment of cultivation parameters can be made. The run is ended manually whenever required.

Questions:

During startup of a run, a ***Questions*** pane is displayed in which questions have to be answered before the run can be started.

No. 1:	Did you calibrate the used pumps with respective tubing?
Question type:	Mandatory, display in chromatogram
Answer type:	Multiple choice

3.2.2 Perfusion culture

ReadyToProcess WAVE 25 (1.2.0.1) or higher, UNICORN 6.3.2 or higher

Method for running cell cultivation in perfusion mode using ReadyToProcess WAVE 25 includes setup of the system, Cellbag bioreactor installation, bioreactor bag inflation, media fill, equilibration, probe calibration, inoculation, pH and DO control, and cultivation phase.

The method uses variables, which are part of an instruction (e.g., a parameter) and has default set points that can be changed during startup of the run in the ***Start protocol variables*** pane.

Note: *This is a template that provides support for setting up a perfusion culture. The user needs to set parameters to set points suitable for the specific process during startup of the run in the* ***Start protocol variables*** *pane. Depending on the specific process, the choice given by the variables might not be enough and changes to the text instructions might be necessary*

Phase ***Method Settings***:

Sets the Cellbag size (selected in ***Variables*** pane during start up) and gives notifications to attach probe cables, install the Cellbag bioreactor, and attach air line and filter heater.

Phase ***Cellbag Inflation***:

Sets the air source and gas flow settings (variable), starts fast fill for inflation, sets the CO2 mix parameters (variable), and notifies the user to connect base / acid bottle for pH control (if required) and to install the lid. Finally, the scale is tared.

Phase ***Media Fill***:

Notifies the user to attach the cell culture media vessel to the Cellbag bioreactor and install the pump tubing, and then starts the media fill up to the set point given in ***Variables***. Holds until the set point has been reached, after which a message informs the user to disconnect the media vessel.

Phase ***Equilibration***:

Starts rocking at the speed and angle defined in ***Variables*** during startup. Starts heating (set point defined in ***Variables***). Duration 2 h.

Phase ***Probe Calibration***:

Notifies the user to perform DO and pH calibration, including pH offline measurement and calibration.

Phase ***Inoculation***:

Notifies the user to attach the inoculation vessel to the Cellbag bioreactor and install the pump tubing. After this step, inoculation is started by running in media control mode up to the required total weight of the Cellbag bioreactor, after which a message informs the user to disconnect the inoculation vessel.

Phase ***Initiate pH and DO Control***:

Start of DO and pH control according to settings defined in ***Variables***.

Phase ***Perfusion***:

Phase with duration of 5000 hours.

Perfusion is started after 72 hours with settings given in ***Variables*** during startup ("weight set point for perfusion" and "perfusion rate").

At 68 hours, a message informs the user that perfusion will start in four hours, with a reminder to prepare and connect feed vessel in time.

At any point during this phase, manual adjustment of cultivation parameters can be made. The run is ended manually whenever required.

Questions:

During startup of a run, a ***Questions*** pane is displayed in which questions have to be answered before the run can be started.

No. 1:	Did you calibrate the used pumps with respective tubing?
Question type:	Mandatory, display in chromatogram
Answer type:	Multiple choice
No. 2:	Aseptically connect suitable harvest vessel to female luer harvest port under laminar flow hood
Question type:	Mandatory, display in chromatogram
Answer type:	Multiple choice

3.2.3 ActiCHO Media System

ReadyToProcess WAVE 25 (1.2.0.1) or higher UNICORN 6.3.2 or higher

Method for running cell cultivation in perfusion mode using ReadyToProcess WAVE 25 includes setup of the system, Cellbag bioreactor installation, bioreactor inflation, media fill, equilibration, probe calibration, inoculation, pH and DO control, and cultivation phase.

The method uses variables, which are part of an instruction (e.g., a parameter) and has default set points that can be changed during startup of the run in the ***Start protocol variables*** pane.

Note: *This is a template that provides support for setting up a perfusion culture. The user needs to set parameters to set points suitable for the specific process during startup of the run in* ***Start protocol variables*** *pane. Depending on the specific process, the choice given by the variables might not be enough and changes to the text instructions might be necessary.*

Phase ***Method Settings***:

Sets the Cellbag size (selected in ***Variables*** pane during start up) and gives notifications to attach probe cables, install the Cellbag bioreactor, and attach air line and filter heater.

Phase ***Cellbag Inflation***:

Sets the air source and gas flow settings (variable), starts fast fill for inflation, sets the CO2 mix parameters (variable), and notifies the user to connect base / acid bottle for pH control (if required) and to install the lid. Finally, the scale is tared.

Phase ***Media Fill***:

Notifies the user to attach the cell culture media vessel to the Cellbag bioreactor and install the pump tubing, and then starts the media fill up to the set point given in ***Variables***. Holds until the set point has been reached, after which a message informs the user to disconnect the media vessel.

Phase ***Equilibration***:

Starts rocking at the speed and angle defined in ***Variables*** during startup. Starts heating (set point defined in ***Variables***). Duration 2 h.

Phase ***Probe Calibration***:

Notifies the user to perform DO and pH calibration, including pH offline measurement and calibration

Phase ***Inoculation***:

Notifies the user to attach the inoculation vessel to the Cellbag bioreactor and install the pump tubing. After this step, inoculation is started by running in media control mode up to the required total weight of the Cellbag bioreactor, after which a message informs the user to disconnect the inoculation vessel.

Phase ***Initiate pH and DO Control***:

Start of DO and pH control according to settings defined in ***Variables***.

Phase ***ActiCHO Feeding***:

In this phase, loops are used for feed shots. Default feed shots are adapted for 10 L working volume and need to be adjusted for other volumes.

After 72 hours, the first feed is given: 400 mL Feed A over four minutes followed by 40 mL Feed B over five minutes. The first loop ends after 24 hours, before the next feeding loop starts. Default number of feeding loops is 20, resulting in 23 days total cultivation time. The number of feeding loops can be changed during startup of the run.

Four hours before the first feeding cycle, a message is displayed to remind the user to prepare and connect feed vessels.

At any point during this phase, manual adjustment of cultivation parameters can be made. The run is ended manually whenever required or will end automatically after the set number of feed cycles (defined in ***Variables***) has been reached.

Questions:

During startup of a run, a ***Questions*** pane is displayed in which questions have to be answered before the run can be started.

No. 1:	Did you calibrate the used pumps with respective tubing?
Question type:	Mandatory, display in chromatogram
Answer type:	Multiple choice

4 Edit methods

In this chapter

4.1 Working with template methods

Open a new template

Follow the instruction below to open a new template.

Step	Action
1	Open UNICORN ***Method Editor***.
2	In ***Method Editor*** module, open the ***Method Navigator***, and double-click on selected method

Method Editor modes

The ***Method Editor*** interface operates in two modes. Select mode by clicking the corresponding icon.

- ***Text Instructions*** editor for entering and editing method instructions
- ***Phase Properties*** for viewing variables (no further options for ReadyToProcess WAVE 25)

4.2 Working with phases and blocks

Phases and blocks

Methods in UNICORN are usually divided into phases. Phases typically represent well-defined steps in the cell cultivation process, such as:

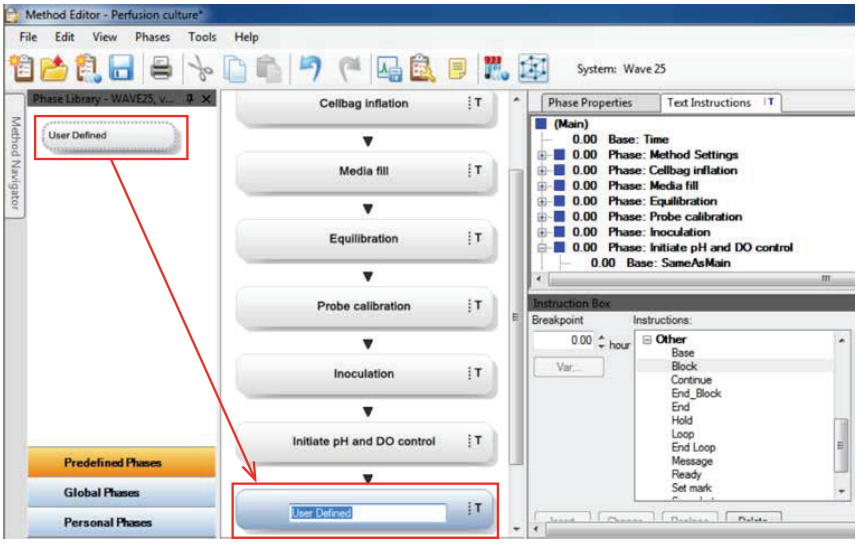
- Cellbag inflation
- Media fill
- Equilibration
- Inoculation
- Cell cultivation

Each phase in a method consists of a series of instructions, which request specific operations in the system. It is possible to further divide a phase using blocks. Every phase and block must start with the ***Base*** instruction and end with the ***End_Block*** instruction, with all specific instructions in between. The instructions are executed in the order they are written until the phase block is finished by the ***End_Block*** instruction. At that time the method will proceed to the next block or phase.

In the ***Text Instructions***, phases and blocks are marked with a blue square.

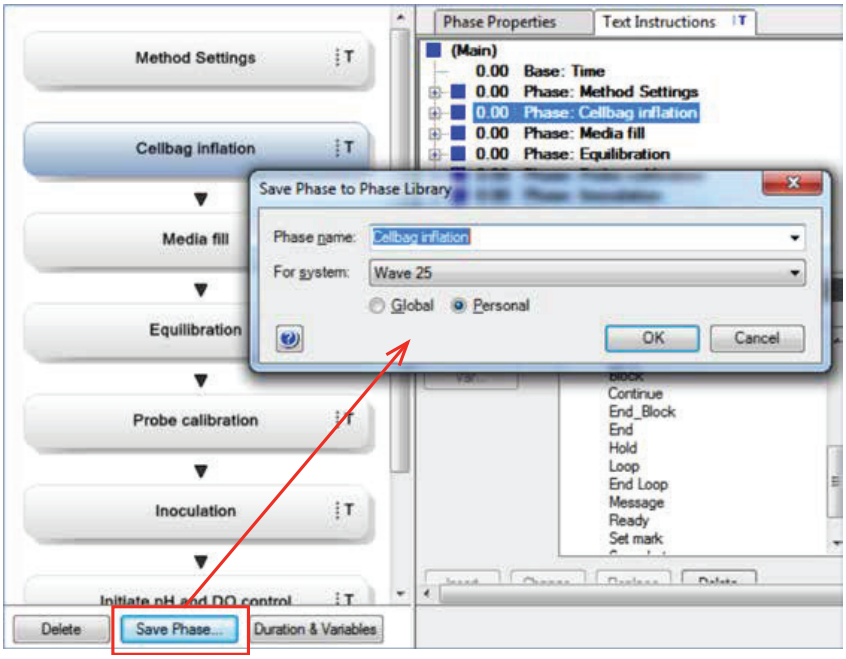
Add a new phase

To add a new phase, select the User Defined phase in the ***Predefined Phases*** section of the ***Phase Library***. Drag and drop the ***User Defined*** box to the required position in the method. Change the phase name by right-clicking on ***User Defined*** and choose ***Rename***.



Save a phase in the Phase Library

To save a phase, select the phase in the ***Method*** and click on ***Save Phase***. The phase can be saved as global (to be used by others as well) or as personal (to be used only by the originator). The phase will then be available in the ***Phase Library*** and can easily be inserted into other methods.

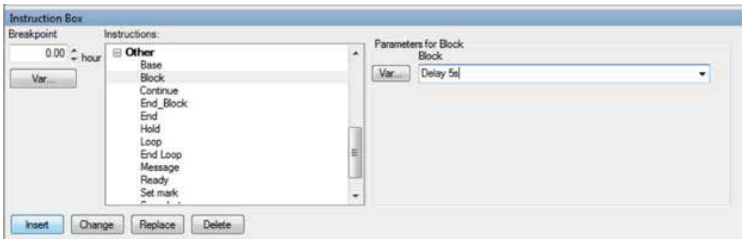


Add a new block

Follow the instruction below to add blocks with the ***Instruction Box***.

Step	Action
1	In the ***Text*** pane of the ***Text Instructions*** editor, select the instruction or block that will precede the new block.
2	In the ***Instruction Box***, select ***Other > Block***.

Step	Action
3	If you wish to add a block, enter a name for the new block in the ***Block*** field, or select the block to insert in the drop-down menu.



Click ***Insert***.

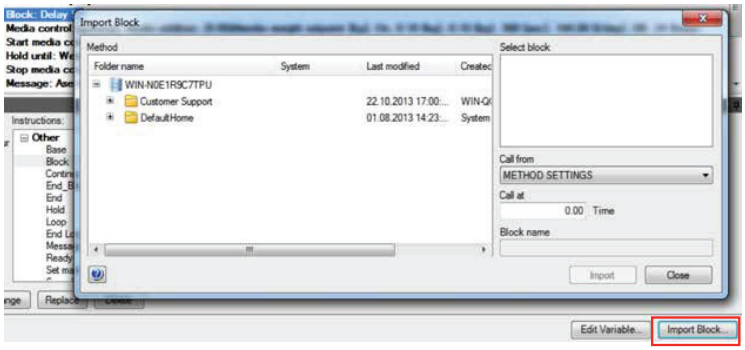
Note:
*A new block creates the framework for the block. The **Base** and **End_Block** instructions are default and additional instructions are added by the user.*

Result:
The new block is inserted right after the block selected in step 1.

Import blocks

Follow the instruction below to import method blocks.

Step	Action
1	In the ***Instruction Box***, click ***Import Block***. *Result:* The ***Import Block*** dialog is displayed.



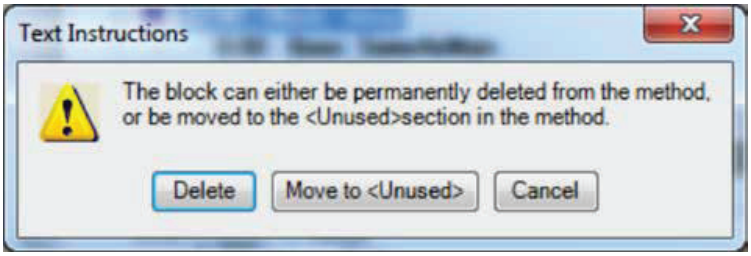
2	Select the method from which you wish to import a block. Select the block to ***import***.

Step	Action
	Result: The name of the selected block is displayed in the ***Block name*** field. You can rename the block from here and import it under a new name.
3	In the ***Call*** field, do the following: In the ***From*** drop-down list, select a block into which the block will be imported. In the ***At*** field, select the break-point value for the block to be imported.
4	Click ***Import***. *Result:* If the selected block contains sub-blocks, the user will be asked if the sub-blocks are to be imported as well. ***Note:*** *An imported block cannot have the same name as an existing block in the method or you will be asked if you wish to replace the existing block. If not, rename the block to be inserted in the* ***Block name*** *field before importing.*
5	Repeat steps 2 and 3 if needed.
6	Click ***Close*** ***Tip:*** *When importing multiple blocks it may be easier to import them all into* ***<Unused>*** *and insert them into the desired locations from the* ***Text Instructions*** *box, see* *Add a new block, on page 18**.*

Delete or move blocks from a method

Follow the instruction below to delete blocks completely from the method or to place blocks in the ***<Unused>*** section of the method.

Step	Action
1	Right-click a block and choose ***Delete*** from the shortcut menu.

Step	Action
	Result: The ***Delete Block*** dialog opens. 
2	Choose from the following options: Click ***Delete***. *Result:* The block is entirely removed from the method. ***Note:*** *If the block contains sub-blocks, another dialog box is displayed asking if you wish to delete the sub- blocks as well.* Click ***Move to <Unused>***. ***Result:*** The block is deleted from the method and transferred to the ***<Unused>*** section of the method. ***Note:*** *If a block is called several times in a method, UNICORN will delete only the block currently marked in the* ***Text*** *pane and will not show the* ***Delete block*** *dialog*

Rename phases or blocks

Follow the instruction below to rename phases or blocks.

Step	Action
1	Right-click the phase or block you wish to rename and select ***Rename***. *Result:* The phase or block name is highlighted in blue and can be changed

4.3 Working with text instructions

Base instruction

Every method block must start with a ***Base*** instruction, defining the base for calculating breakpoints. Different blocks in the same method may be written on different bases.

Method blocks for cell cultivation are entered in base time "hours".

Tip: *For all blocks other than the main block, the base may also be defined as* ***SameAsMain****, which means that the block will inherit the base defined in the main block.*

Change an instruction

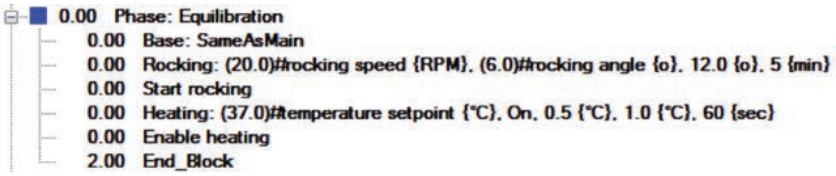
Follow the instruction below to change an instruction in the ***Text*** pane ***Text Instructions***.

Step	Action
1	Select the instruction. *Result:* The instruction, with its current parameters, is displayed in the ***Instruction Box***.
2	Make the required changes to the breakpoint or parameters, or select a new instruction in the ***Instruction Box***.
3	Click ***Change*** or ***Replace***. ***Note:*** *The* ***Change*** *and* ***Replace*** *buttons are equivalent unless changes are made to a breakpoint. See the Section below.*

Breakpoints

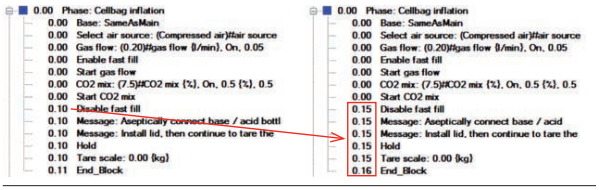
Each instruction in a method is issued at a specified breakpoint according to the base instruction. The first instruction in a phase or block is always at breakpoint 0.00. All other breakpoints are counted from this starting point.

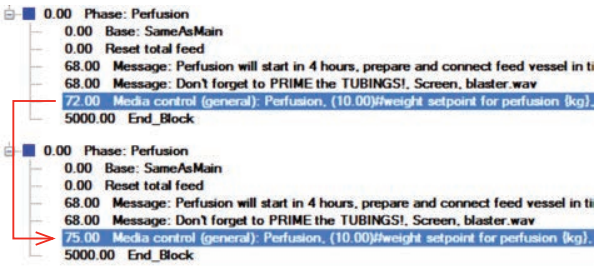
For example, the rocking and heating parameters are set and assigned to be executed at breakpoint 0.00 in the ***Equilibration*** phase. Although the phase ends after two hours (breakpoint 2.00), the rocking and heating will continue as ***End_Block*** only tells UNICORN to go to the next step in the method.



Update breakpoints

The table below describes the difference in functions between ***Change*** and ***Replace*** when you modify breakpoints.

Button	Function
Change	This function shifts all subsequent instructions in the block according to the change in the breakpoint. ***Change*** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in a block. The illustration below shows an example where instruction ***Disable fast fill*** is changed from breakpoint 0.10 to 0.15 hours using ***Change***. All subsequent instructions will be updated accordingly by addition of 0.05 minutes. 

Replace	This function moves the selected instruction but does not change the breakpoint of any other instruction. With ***Replace***, the relative order of the instructions in a method can be changed. The illustration below shows an example where instruction ***Media control: Perfusion*** is moved from breakpoint 72.00 to 75.00 hours using ***Replace***. No other instruction is affected by this action. 

4.4 Working with variables

Variables

All template methods use variables. Each variable indicates an option for user choice. Variables allow one method to be used for runs under a variety of conditions, such as different inoculation volumes. Variables can be assigned to most instruction parameters including breakpoints.

Note: *In validated environments, the use of variables may be minimized or eliminated once the final method has been verified.*

Two types of variables

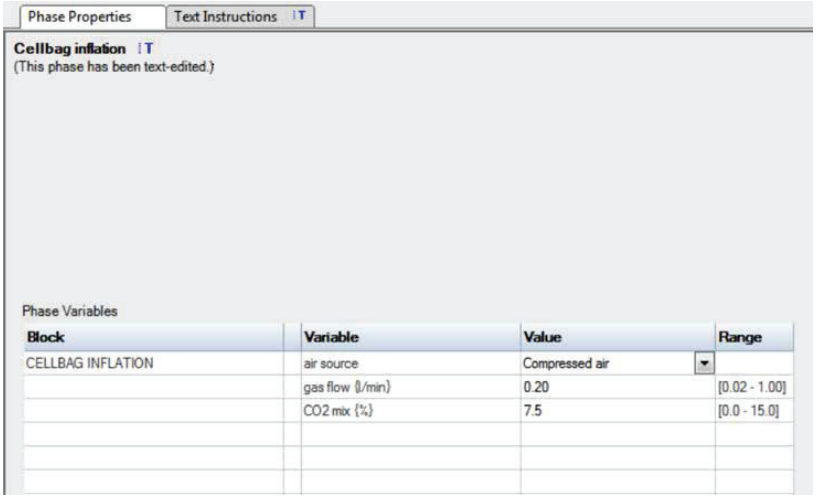
There are two types of variables that can be assigned to instructions, see the Table below.

Variable type	Description
Parameter	Defines the function of the instruction, such as rocking speed and angle, or the gas flow and temperature set points.
Breakpoint	Defines when the instruction occurs and how long it will last.

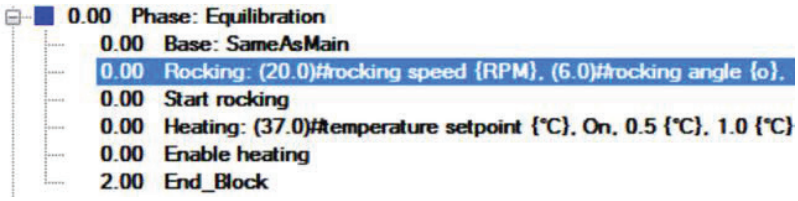
Identify variables

Variables in a method can be identified in several ways:

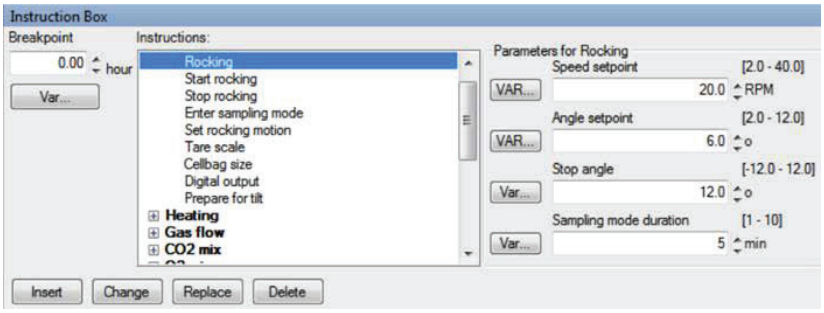
- All variables of a selected phase are listed and can be modified in the ***Variable List*** under ***Phase Properties***.



- In the ***Text*** pane of ***Text Instructions***, the values of the parameter variables are shown in parentheses followed by the variable name preceded by "#".



- In the Instructions field of the ***Instruction Box***, parameter variables are indicated by the ***VAR*** (not ***Var***) button in parameter field.



Change variable values

To change default variable values, you can either

- edit the instruction or block in the ***Instruction box*** of ***Text Instructions***, or
- change the value in the ***Variables List*** under ***Phase Properties***, or
- change the value in the ***Variables*** dialog of the ***System Control*** module immediately before the start of a run without using the ***Method Editor***

Variable names

Variables are defined with names, which can be explicit descriptions of the variable function, for example, ***media plus inoculum set point***. Suitable choice of variable names can make the method easier to read and understand, and also help the operator in setting variable values at the start of a run.

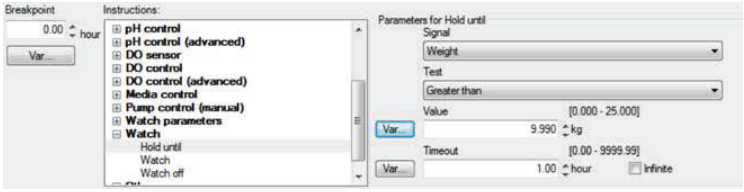
The variable name can be up to 50 characters long and all characters are accepted except for the following:

- *?/\:|<>" Variable names are case-sensitive and names such as ***gas flow*** and ***GAS FLOW*** will be treated as two different variables.

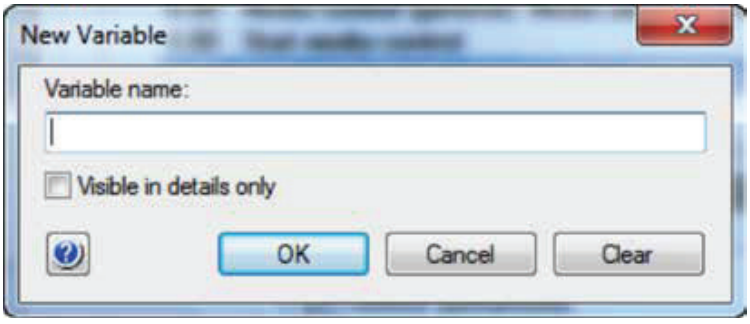
Define variables

Within each block, only one variable affecting the block length may be defined. However, any number of parameters may be defined as variables within a block. As many as 64 parameters can be defined as variables in an entire method. Each parameter defined as a variable will be assigned a default value, which is used if no changes are made of the value at the start of the run. Follow the instruction below to define a new variable.

Step	Action
1	Select the instruction, in which you wish to define the variable, in the ***Text*** pane of ***Text Instructions.*** *Result:* The parameters for the instruction are shown in the ***Instruction Box.***



2	Locate the breakpoint or the parameter in the ***Instruction Box***. Click ***Var***. *Result:* The ***New Variable*** dialog opens.



3	In the ***New Variable*** dialog: Enter a variable name. Check the ***Visible in details only*** box if you wish to set the variable as a detailed variable. Detailed variables only become visible under the ***Variables*** tab if the ***Show details*** check box is selected. Click ***OK***.

Step	Action
	Result: The ***Var*** button changes to ***VAR*** to confirm the new variable. The variable is displayed in the ***Text*** pane.

Note: *UNICORN 6 supports global variables. By defining a variable a name that already exists (for the same parameter in the same instruction) in a method, the value for the variable will be updated wherever that variable name is used.*

4.5 Working with conditional instructions

Watch instructions

Watch instructions enable execution of a specific action when a specified monitor signal value for a given condition is met. There are ***Watch*** instructions for each process monitor signal and each Watch instruction can use different conditions to respond to a signal value. Each ***Watch*** instruction must also be assigned an action to be executed once the condition is met, and a breakpoint for the ***Watch*** instruction to start.

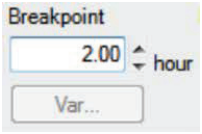
When is a *Watch* instruction active?

The breakpoint when the ***Watch*** instruction is issued determines when the watch begins, not when the block is activated. Once set, a ***Watch*** instruction remains active until:

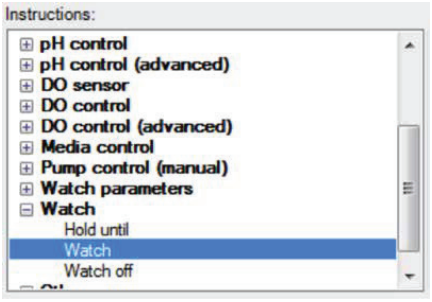
- the condition is met, or
- a new ***Watch*** instruction is issued for the same monitor signal, or
- the ***Watch*** instruction is turned off through a ***Watch_off*** instruction

Insert a *Watch* instruction

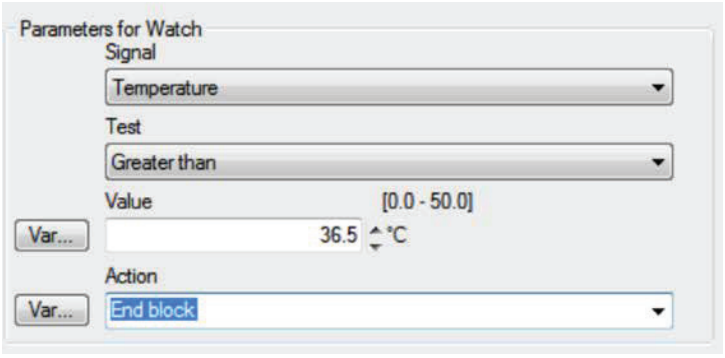
Watch instructions are inserted in the ***Instruction Box*** of the ***Text Instructions Editor***. Follow the instruction below to insert a ***Watch*** instruction.

Step	Action
1	In the ***Breakpoint*** field, select the appropriate breakpoint. The breakpoint determines when the watch begins. 
2	In the ***Instructions*** field: Select ***Watch***.

Step	Action



3 In the ***Parameters*** field, select appropriate values under ***Signal***, ***Test***, ***Value***, and ***Action***.



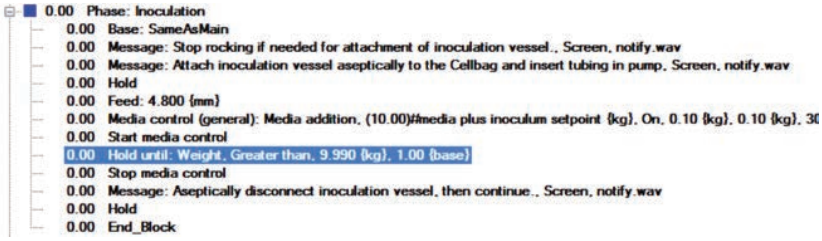
4 Click ***Insert***.

Result:
The new ***Watch*** instruction is inserted into the method at the selected breakpoint .

Hold_Until instructions

The ***Hold_Until*** instruction is a special kind of ***Watch*** instruction. The method is put on hold until a specific condition (signal, test and value) is met or a specified ***Timeout*** is reached and the method execution can be continued. Instructions at the same breakpoint as the ***Hold_Until*** instruction, but that are placed after the ***Hold_Until*** instruction, will be executed after the method execution is continued.

In ReadyToProcess WAVE 25 template methods, ***Watch*** instructions are used in the phases ***Media Fill*** and ***Inoculation*** to stall the method until media or inoculum has been filled into the Cellbag bioreactor.

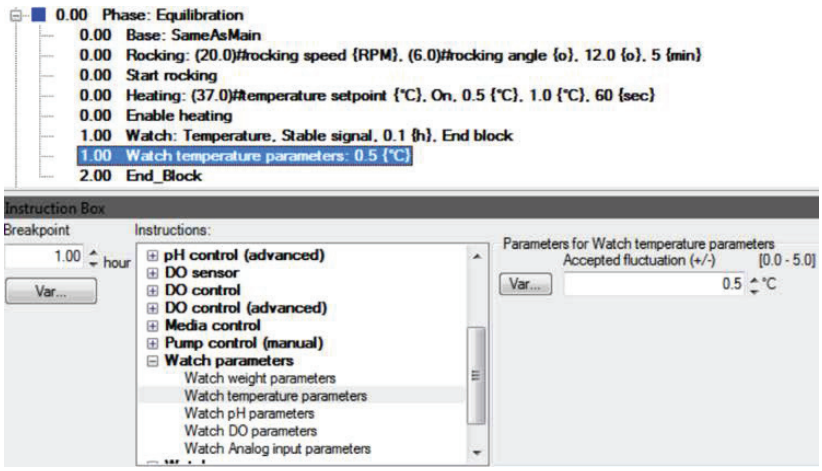


Watch for stable signal

The condition defined in ***Watch Stable signal*** is met if the signal does not deviate more than defined under ***Accepted fluctuation (+/-)*** (see *Watch parameters, on page 31*) from the signal at the start of the watch during the time interval specified for the watch. If the condition is not met, a new watch interval is started, with a new value defined by the signal level at the start of the new interval.

Watch parameters

Accepted fluctuation of the signal you wish to watch can be defined under ***Watch parameters*** and will affect ***Watch Stable signal***. For ***Watch Stable signal*** to be executed correctly, you need to activate both the ***Watch Stable signal*** and the ***Accepted fluctuation*** instruction.



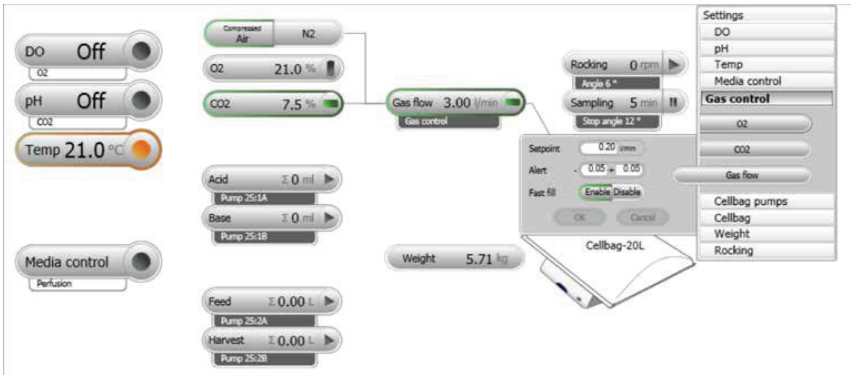
5 During a run

Buttons in System Control

Button	Function
	Clicking ***Run*** opens the ***Run*** dialog, which shows all available methods. If a method is loaded, ***Run Setup*** opens.
	Clicking ***Hold*** suspends execution of the method, while all settings, such as rocking and temperature, will remain.
	Clicking ***Continue*** resumes the execution of a held method.
	Clicking ***End*** terminates the method execution and puts the system into an end state.

Manual interaction

During a run, the user can manually change settings of the cultivation (rocking speed and angle, gas flow, etc.) from the process picture.



6 Reference information

Literature

For further information on the ReadyToProcess WAVE 25 system, please refer to the following documents:

- ReadyToProcess WAVE 25, Operating Instructions (29009597)
- ReadyToProcess WAVE 25, Product Documentation (29065057)
- ReadyToProcess WAVE 25, System Handbook (29009598)

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