BelloStage™ Operation Manual

Technical communication document including the guide and associated images to give assistance to the user in running the BelloCell® system.

ESCO LIFESCIENCES GROUP

ESCO Bioengineering Co., Ltd.

Contents

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Disclaimer Notice

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Warranty

EBE warrants that if a product manufactured by EBE shall be free of defects in materials including mechanical parts and electronic parts and other workmanship for one (1) year from the first to occur of (i) the DATE the product is sold by EBE directly or (ii) the DATE the product is purchased from authorized distributors by EBE (the “Commencement DATE”).

Except as expressly stated above, EBE makes no other warranty, expressed or implied, with respect to the products and expressly disclaims any and all warranties, including but not limited to, warranties of design, merchant ability and fitness for a particular purpose.

An authorized distributor of EBE must perform all warranty inspections. In the event of defect covered by EBE's warranty, EBE shall, as its sole obligation and exclusive remedy, provide free replacement parts to remedy the defective product.

This instrument warranty is not allowed to be transferable. The warranty only applies to the original purchaser of this instrument and will be null and void in the case of resale, and possible re-location.

This warranty shall be invalid due to damage caused by accident, misuse, theft, neglect, natural disaster, use of non-EBE's authorized spare parts, illegal disassembly, etc., relocation or deterioration caused by application which is not the original purpose to design this product.

Caution

Users, if necessary, have to wear gloves, goggles and suitable protection clothes before operating devices.

This equipment must be operated as described in this manual. If operational guidelines are not followed, equipment damage and personal injury can occur.

Please read the entire User's Guide before attempting to use this unit.

Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.

EBE is not responsible for any damage to this equipment that may result from the use of an

accessory not manufactured by EBE.

Return of Material Authorization Policy

EBE Technical Support Team:

info@escobioeng.com, mail@vaccixcell.com

If service is required, please contact our technical support team.

Please do not return any equipment for service without a Return Authorization and Number, which can be obtained from our technical support team.

The return authorization number must appear on the outside of all cartons.

Item	Description
Customer Company Name	
Customer Name	
Customer Phone Number	
Purchase Date	
Machine Serial Number	
Invoice Number	
Reason for Returning Back	

Chapter 1 BelloCell® System Brief Introduction

1.1 Scope

This document covers the installation, operation, and maintenance of BelloCell® System. The BelloCell® System is a product designed and manufactured by EBE and is used for cell culture with BelloCell® bottle series.

1.2 Introduction

1.2.1 BelloCell® System

BelloCell® System includes a control panel and the main body. In the main body, the internal platform, which is used to compress the bellow of BelloCell® bottle, is driven by a step motor and guiding screw. When the internal platform moves upward, the bellow of BelloCell® bottle is compressed and therefore the matrices are submerged in the ascending medium. Once the internal platform moves downward, the medium flows down and the matrices are exposed to the air.

The ascending (up) rate, descending (down) rate, top holding time, and bottom holding time can be adjusted while the machine is running (without pausing). **While removing BelloCell® bottle from BelloStage™, please press the button of "STOP" and wait till the internal platform going back to its initial position.** And then take out the BelloCell® bottle.

Please remember to place the control panel on the outside of the incubator.

1.2.2 BelloCell® 500 Series Bottle

BelloCell® bottle includes the culture chamber and the plastic bellow. The upper chamber is allowed the packing of matrix BioNOC™ II or BioMESH™ for the cell culture. The medium in the bellow will rise when the bellow is pushed up by BelloStage™, causing the BioNOC™ II or BioMESH™ to submerge in the medium. The periodical submerging and aeration of the matrix will lead to the fast and sufficient nutrition-waste exchange and oxygen supply, creating an optimal environment for the cell growth.

Chapter 2 Inspection and Unpacking

2.1 Inspection of Boxes

After you have received your order from EBE inspect the boxes carefully for any damage that may have occurred during shipping. Report any damage to the carrier, EBE, your local distributors or dealers immediately.

2.2 Packaging Verification

Unpack your order, saving the packing materials for possible future use. Also, be sure to save the User's Guide, for instruction and reference.

Verify against your EBE packing list that you have received the correct materials, and that nothing is missing. The part numbers on the packing list correspond to part numbers and/or manufacturing numbers. You will find the number affixed to each item, either on paper sticker on the packaging or on the serial number identification sticker on the equipment itself.

If any part of your order was damaged during shipping or was missing, or fails to operate, please fill out Customer Satisfaction Form and return it by fax or mail, to obtain a Return Shipment Authorization Number. You can also contact our Technical Support Team.

Chapter 3 Preparation of Location

3.1 Physical Location

The surface where you place BelloCell® System should be smooth, level and sturdy. Ensure that there is enough space around the back and the front of the system for proper operation and access.

3.2 Environment

BelloCell® System should work under：

- 20 °C to 42 °C ambient temperature.
- Relative humidity up to 90%.

3.3 Utility

With the AC transformer provided, the BelloStage™ can run on 100-240 Volts, 50 or 60 Hz. You may require an additional adapter to mate the transformer plug to your electrical supply

outlet.

- Gas Source: Controlled by CO_2 incubator
- Power Source: AC100-220V 50/60Hz

Chapter 4 Safety Information

- Please read this entire instruction manual prior to use the system.
- BelloStage™ operates on 100~220 volts, 50/60 Hz through a desktop AC transformer.
- Use a properly grounded electrical outlet of correct voltage and current handling capacity.
- Please apply the lubricating grease on the ball screw every three (3) months or as necessary
- Please do not place the control panel of BelloStage™ inside the incubator.
- Avoid splashing any liquid on the BelloStage™ or submerging it.
- Avoid inserting your hands inside the body of BelloStage™ during operation.
- Please do not open the adapter.
- For the consumables of BelloCell® system (BelloCell® bottles), please use them as single-use disposables. Once the culture process is complete, appropriate decontamination is required prior to the disposal.
- BelloCell® system is for laboratory or manufacture use only. It is not intended for diagnostic or therapeutic use in humans or animals.
- A BelloCell® bottle is one-time use only. When the experiments are finished, BelloCell® Bottle should be decontaminated and discarded. Consult local institution for detail.

Chapter 5 BelloCell® System Introduction

5.1 System Identification

Item	Description
System Name	BelloCell® System
Manufacturer	ESCO Bioengineering Co., Ltd.
PO/Invoice Number	
System Serial Number	

5.2 Specification

5.2.1 Core Module of BelloStage™

Item	Description	Specification
Infrastructure	Exterior: Main body Control panel	W x D x H (mm): 280 x 375 x 180 226 x 137 x 40
	Weight Main body Control panel	(kg): 11.0 1.2
	Power	AC 100~220 V 50/60 Hz, 12 Amp
	Motor	DC step motor
	Sensor	Micro-switch sensor
	Material	SS304L
Function	Ascending Rate	0.25 - 2.0 mm/sec Interval: 0.25 mm/sec
	Descending Rate	0.25 - 2.0 mm/sec Interval: 0.25 mm/sec
	Top Holding Time	0 - 99 min 59 sec Interval: 1 sec

	Bottom Holding Time	0 - 99 min 59 sec Interval: 1 sec

5.2.2 BelloCell® bottle

Item	Name	Specification
Infrastructure	Exterior	243(h) x 50(r) mm
	Effective Volume	500 ml
	Matrix	BioNOC™ II, 100 ml (850 pieces, ±5%) BioMESH™, 100 ml (360 pieces, ±5%)
	Air Filter	PTFE membrane, 0.22 μm
	Material	PETG, LDPE/EVA, PP

5.3 Shipped Part List

Item	Description	Quantity
BelloStage™ main body	BelloCell® Bottle Placement	1
Control panel	Set up parameters for ascending/descending rates and top/bottom holding time	1
Power cord	Connect to the power supply	1
Signal cable	Connection between main body and control panel	1
Forceps	For matrix sampling	2
CVD (crystal violet dye)	Fast cell disruption and nuclei staining for nuclei count	1
Lubricating oil (SR1)	For lubrication	1

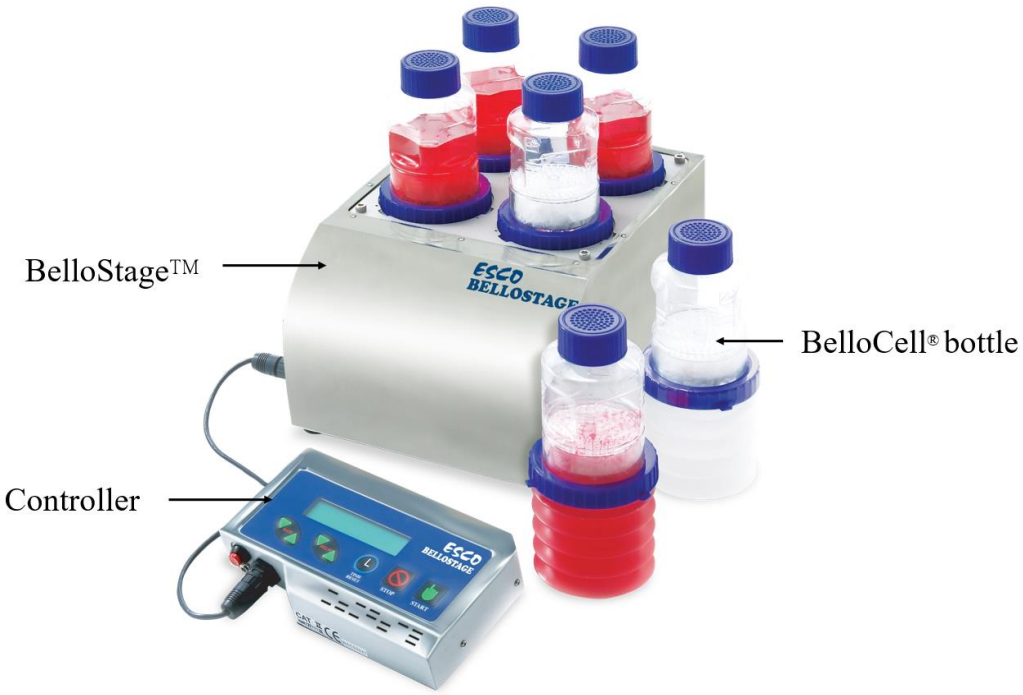
5.4 System Construction Description

5.4.1 Overall View

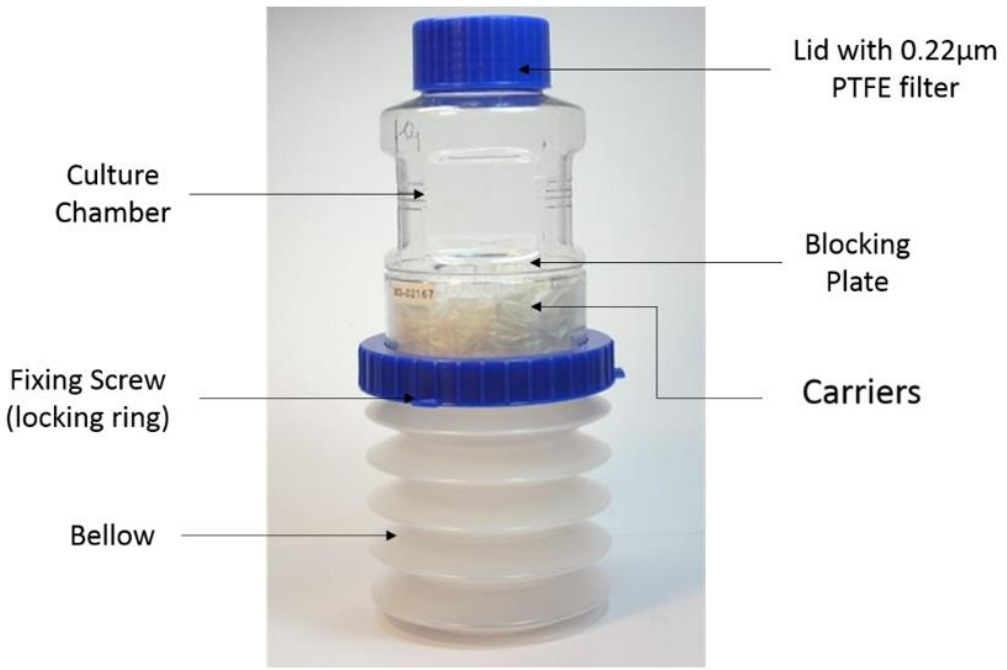
BelloStage™ is part of the BelloCell® Culture System. The unit, which is capable of holding up to four disposable cell culture bottles (BelloCell® 500 Series Bottles) and fits easily inside the culture incubator, while the magnetic control box attaches conveniently to the outside metal wall of the incubator for easy access. By an internal platform compressing and relieving the bellow of the bottle, it renders the medium in the bellow up and down according to the set program and optimizes oxygenation as well. As the platform lifts, it compresses the bellows, enforcing the medium into the chamber that contains the BioNOC™ II or BioMESH™ matrices from the bellow; as the platform descends, the media returns to the expanding bellows, which in turns exposing the matrices to the atmospheric environment.

BelloCell® 500 Series Bottle is made of disposable plastic materials. BioNOC™ II or BioMESH™ matrices are packed in the upper chamber of a BelloCell® Bottle unit. Animal cells are immobilized within carriers and the culture medium is moving up and down between the upper chamber and the bellows by BelloStage™.

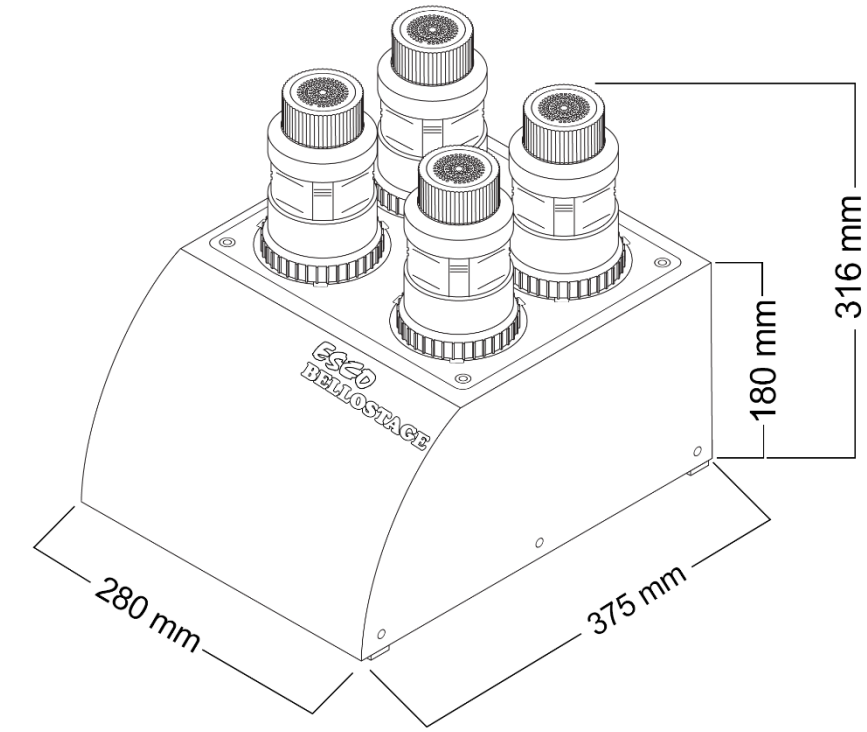
5.4.2 BelloCell® System

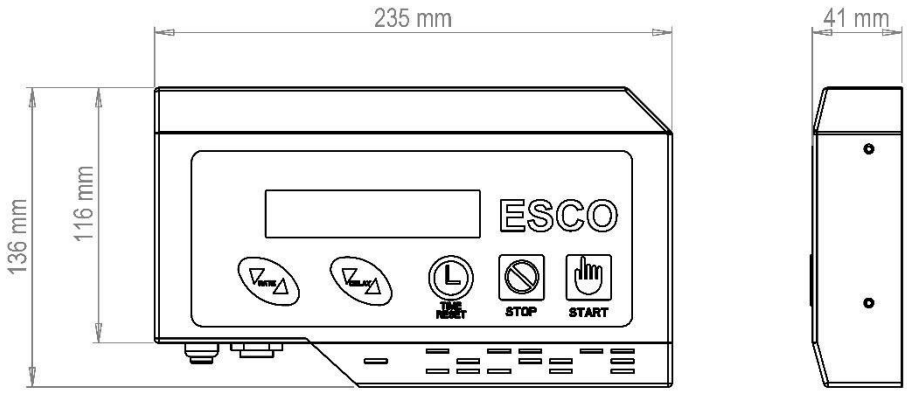


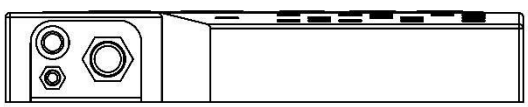
5.4.3 BelloCell® Bottle

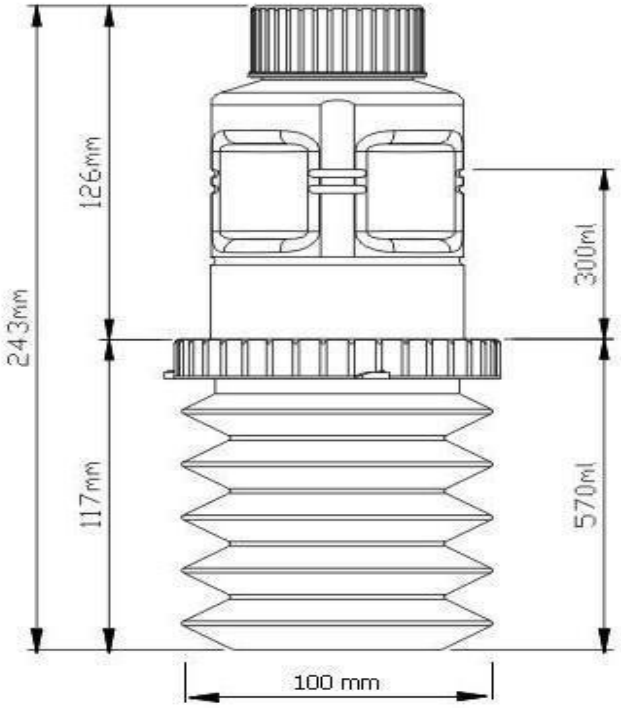


5.4.4 Dimensions



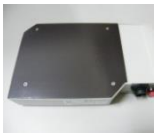






5.5 Functional Description

5.5.1 Control Panel

Item	Description
	Power button for the control panel.
	9-pin socket for the signal cable connected to the main body of BelloCell® System.
	The socket for the power cord of the control panel.
	Magnetic patch on the back of the control panel, enabling the control panel to attach on the door of the incubator.

	Start the upward and downward movement of the internal platform of BelloStage™ main body.
	Pause the upward and downward movement of the internal platform. Switch between “T_H (Top Hold)” and “B_H (Bottom hold)”
	Switch between “Version (v6.1)”, “Alert Mode (Continuous)” and “Total time: 000hr00min” (Culture process). Press the button for 3 sec to zero the total time.
	Set up the “Top Holding Time” and “Bottom Holding Time”. Maximum= 99 min 59 sec, interval=1 sec.
	Set up the rate of upward movement (ascending) and downward movement (descending) of the internal platform. Maximum= 2.0 mm/sec, interval= 0.25 mm/sec.

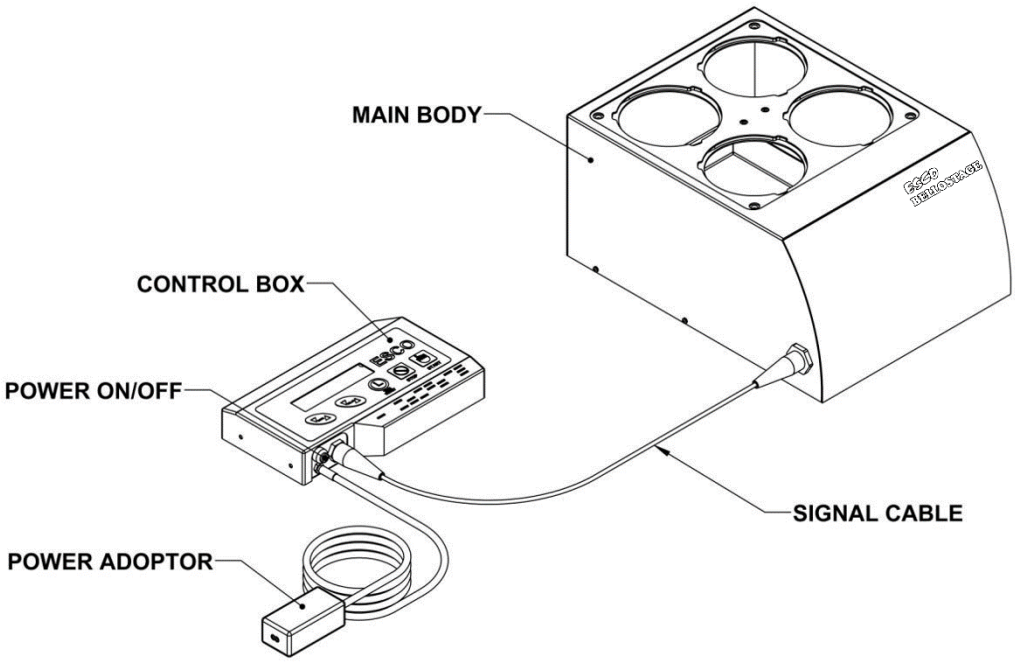
5.5.2 Main Body

Item	Description
	9-pin socket for the signal cable connected to the control panel.
	Tray for disposable; collection of accidental medium leakage.

5.6 Operation Instruction

5.6.1 Preparation

- Carefully remove BelloStage™ main body and the control panel from the carton boxes.
- Place the main body in the CO_2 incubator and the control panel on the door (outside) of the incubator. There is a magnetic patch on the control panel therefore the control panel can attach on the door of the incubator.
- Plug one end of the signal cable on the left side of the main body, pass the cord through the access port of the CO_2 incubator and plug the other end on the control panel. Screw the terminal sockets to lock.
- Plug the power cord to the control panel and then plug the adapter to the power supply.
- Press the power button of BelloStage™. The machine is ready for use.



Warning

- Please do not store BelloStage™ in the incubator for a long time without running it.
- Avoid scraping the surface of the main body.

5.6.2 Basic requirement for cell culture with BelloCell® 500 Series Bottle

Item	Quantity
BelloCell® 500 Series Bottle	1
BelloStage™	1
Incubator (37 °C or depends on cell types)	1
Culture medium	500 ml
Glucose meter or biochemical analysis system (EBE GlucCell Glucose meter, YSI or NOVA or glucose kit)	1
Crystal violet dye nucleus count kit	1 bottle
Long-arm forceps	1, sterilized
T-flasks, pipette, pipette-aids	As needed
Centrifuge	1
Phase contrast Microscope	1
Cell culture reagents (trypsin, PBS,...)	As needed

5.6.3 Basic Operation (Parameter Setup)

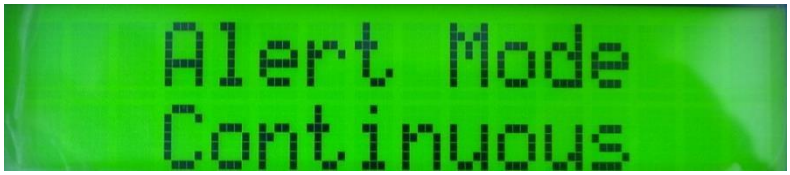
- Once the power button on the control panel is pressed, the internal platform of the main body start to move upward and then downward one time, determining its maximum movement distance (warm up).

- Set up the inoculation parameters by the control panel. The display should show “Up” and “T_H” parameters.

- If not, press "STOP ⊘" once until the "Up" and "T_H" (move upward and top holding) parameters appeared.
- Press ◤RATE◢ to set the "Up" parameter (◤◢) to 2.0 mm/sec (there are 0.25 mm/sec, 0.5 mm/sec, 0.75 mm/sec, 1.0 mm/sec, 1.25 mm/sec, 1.5 mm/sec, 1.75 mm/sec, 2.0 mm/sec available).
- Press ◤DELAY◢ to set the "T_H" parameter (◤◢) to 20 sec (there are from 0 min 0 sec to 99 min 59 sec available).
- Press "STOP ⊘" again to convert to the "Down" and "B_H"" (move downward and bottom holding) parameters.

- Press ◤RATE◢ to set the "Down" parameter to 2.0 mm/sec.
- Press ◤DELAY◢ to set the "B_H" parameter to 0.0 sec.
- To change the time (sec), press ◤DELAY◢ shortly. To change the time (min), "long press" ◤DELAY◢.
- Press "START" to start running. Press "STOP⊘" to stop the function.
- Press "TIME RESET" to switch between "Total Time", "Alert Mode" and "Version". At the page of "Total Time", long press "TIME RESET" for 3 sec to reset (zero) the accumulated running time (total time). Moreover, at the page of "Alert Mode", press

◤DELAY◢ to select “30 seconds” (alarm issues for 30 sec) or “Continuous Mode” (alarm keeps beeping). Press “STOP⃠” to stop beeping.



5.6.4 Basic Operation (Parameter Setup during the run)

- During the run, it is allowed to press ◤RATE◢ to adjust the movement rate of the internal platform.
- It is allowed to press ◤DELAY◢ to adjust the holding time of the internal platform. During the run, it is not allowed to change the ascending rate until the internal platform moves upward; it is not allowed to change the descending rate until the internal platform moves downward.
- During the run, it is not allowed to change “Total Time” and “Alert Mode”.

5.6.5 Basic Operation (Termination of the Run)

- Once pressing “STOP ⃠”, the internal platform will return to the initial position while the display shows “Return to Base. Please wait…”. 2 beeps appear and then the internal platform stops.

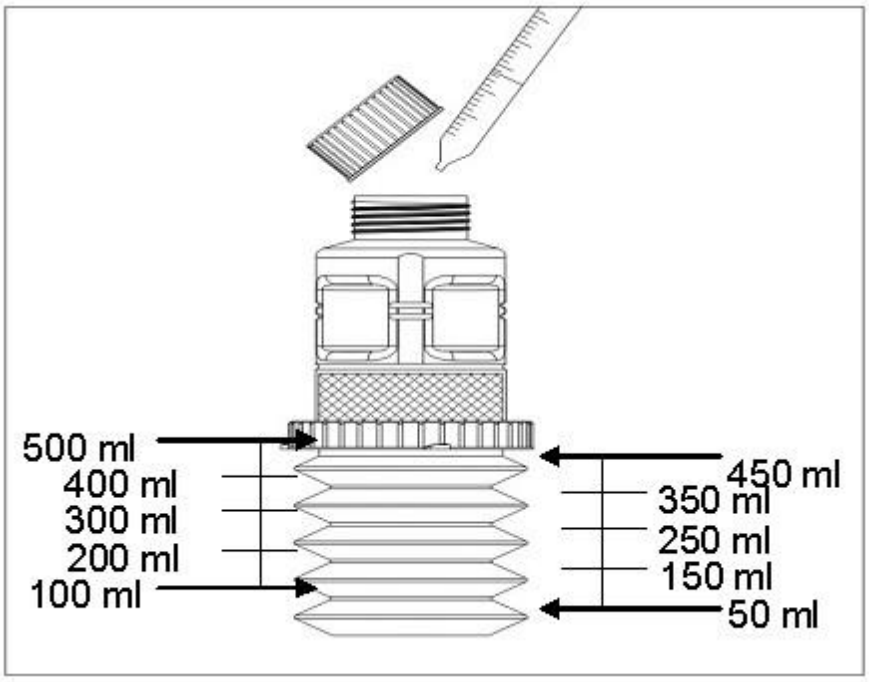
5.6.6 Basic Operation (Alarm)

- If the signal cable is disconnected, the following error message shows.

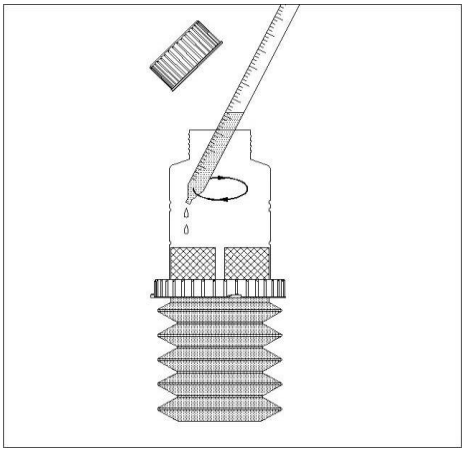
5.6.7 Inoculation and cell culture

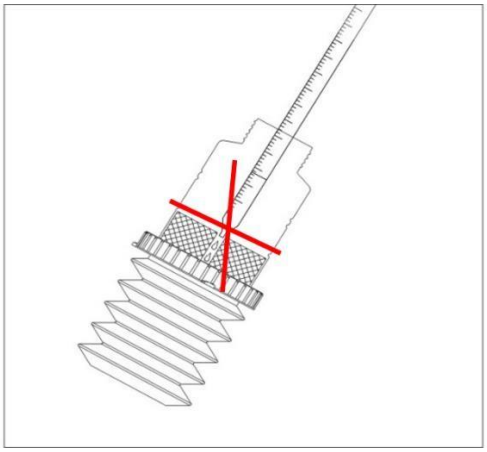
5.6.7.1 BelloCell® 500/500P Bottle

- Take out one BelloCell® 500/P Bottle unit from the package (it is sterilized by gamma irradiation). Place the BelloCell® 500/P Bottle unit in a biological safety cabinet. Open the cap and fill in 450-470 ml (depending on seeding volume) pre-warmed culture medium to the bellow aseptically. Tilt the bottle to send all the media down into the bellow chamber.



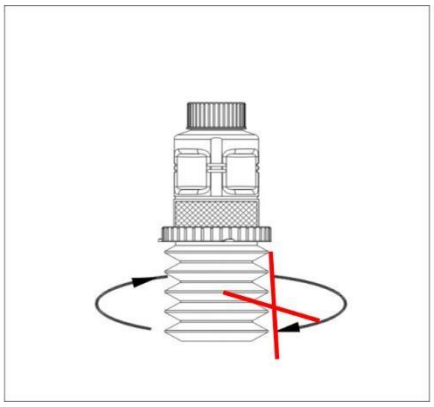
- Dispense 30-50 ml of cell suspension containing a total of 1 x 10^8 ~ 2.0 x 10^8 cells directly on the top of matrices in a clockwise direction. (Please do not add cells into the hole in the middle, otherwise the cells may fall directly onto the bottom of the bottle). Close and tighten the cap. Bring the bottle onto BelloStage™. (Cell seeding density may differ depending on the cell line)

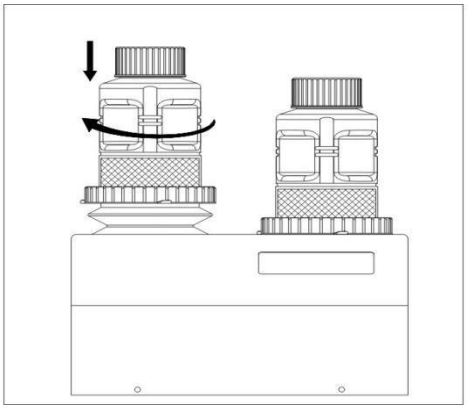




- Fix the BelloCell® 500 Bottle, using the blue wide screw of the bottle into BelloStage ™ main body, and make sure the blue protrusion locks in place. Do not shake the bottle

to avoid the cell attachment. Set up inoculation parameters: Up: 2.0 mm/s, T_H: 20 s; Down: 2.0 mm/s; B_H: 0 s and then press “Start” to initiate the immobilization process. (Change “Up” to “Down” by pressing “STOP” key; to set minutes, press and hold either “DELAY” arrow key; to set seconds, press either “DELAY” arrow key in short bursts.)





- After 2~5 hours, directly set up the moving rate and holding time parameters for cell growing phase without stopping machine.

  Note: pH is critical at this point. After inoculation, make sure that any added media has a pH of 7.0~7.4 if the media contains sodium bicarbonate. CO_2 concentration should be maintained between 5~10% in the incubator. Abnormal pH range will reduce the immobilization efficiency.

- It is recommended to set UP/DOWN rate of 1.0~1.5 mm/sec and TOP/BOTTOM HOLD of 0/1 min for CHO, BHK, Vero, HEK293, and C127 cell lines and UP/DOWN rate of 1.5~2.0 mm/sec and TOP/BOTTOM HOLD of 0/1 min for Sf-9, Hi-5 in the beginning of the run. Please add up initial glucose and glutamine concentration to 3 g/L and 4 mM.

  Note: Increasing the top-holding time allows more nutrient exchange between bulk medium solution and matrix. However, increasing the top-holding time may also reduce the oxygen level in the medium. In addition, increasing the bottom-holding time will

increase the dissolved oxygen level during the cell culture and can also help stabilize the value of pH.

- To increase the mixing efficiency and oxygen supply, these parameters can be modulated depending on user's demand.

Note: Please do not spray the alcohol on the lid of BelloCell® 500 Bottle since there is the air filter on the top of the lid.

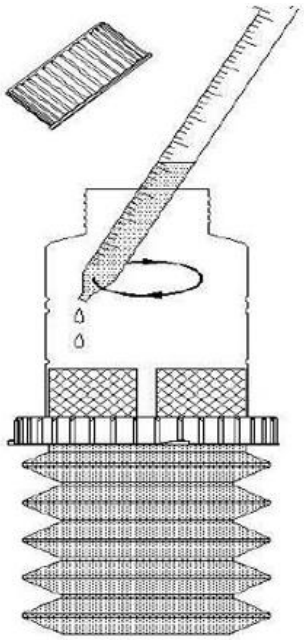
Note: All operation steps have to be done aseptically.

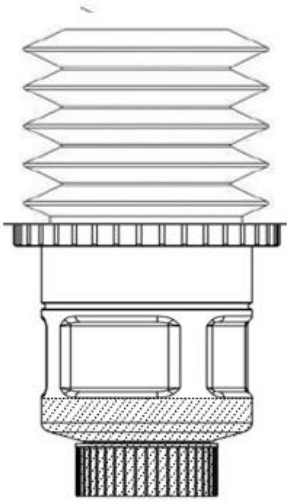
5.6.7.2 BelloCell® 500A/500AP Bottle

- Take out one BelloCell® 500A/AP Bottle unit from the package (it is sterilized by gamma irradiation). Place the BelloCell® 500A/AP Bottle unit in a biological safety cabinet. Take out one BelloCell® bottle unit from bag. Open the cap and fill in **120 ml pre-warmed culture medium aseptically.** Tilt the bottle to send all the media down into the bellow chamber.

Note: The type of BelloCell® bottle and the amount of culture medium to be used varies on the application of the user.

- Dispense 20 ml cell suspension containing a total of **$1.0x10^8$ ~ $3.0x10^8$** cells directly on the top of carriers in a clockwise direction. Replace the blue cap with a white cap and place the upside-down bottles inside CO2 incubator for inoculation. Swirl the bottles periodically to re-distribute the cells and after inoculation for 3-4 hours, fill in 360 ml pre-warmed culture medium and replace the white cap with a bule cap. Close and tighten the cap. Bring the bottle onto BelloStage™ and start operation IMMEDIATELY. (Cell seeding density may differ depending on the cell line)





- After 3~4 hours, set up parameters to Up: 1.0 mm/s, T_H: 0 s, Down: 1.0 mm/s, B_H: 1 M for cell culture (the parameters may vary depending on cell line). Push “Start” (Finger) button to progress culture procedure.

  Note: pH is critical at this point. After inoculation, make sure that any added media has a pH of 7.0~7.4 if the media contains sodium bicarbonate. CO_2 concentration should be maintained between 5~10% in the incubator. Abnormal pH range will reduce the immobilization efficiency.

  Note: Increasing the top-holding time allows more nutrient exchange between bulk medium solution and matrix. However, increasing the top-holding time may also reduce the oxygen level in the medium. In addition, increasing the bottom-holding time will increase the dissolved oxygen level during the cell culture and can also help stabilize the value of pH as well.

- To increase the mixing efficiency and oxygen supply, these parameters can be modulated depending on user’s demand.

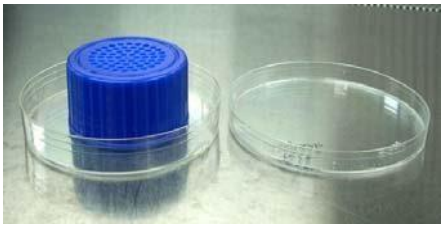
Note: Please do not spray the alcohol on the lid of BelloCell® 500 Bottle since there is the air filter on the top of the lid.

Note: All operation steps have to be done aseptically.

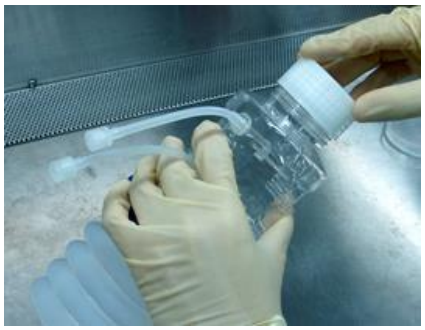
5.6.7.2.1 Bottom-up Operation for Cell immobilization or Virus Infection/transfection

- Remove the vented cap (blue) from the BelloCell®-500AP bottle.

- Place the vented cap in a sterile petri dish and cover the dish to ensure the sterility of the cap for future use.



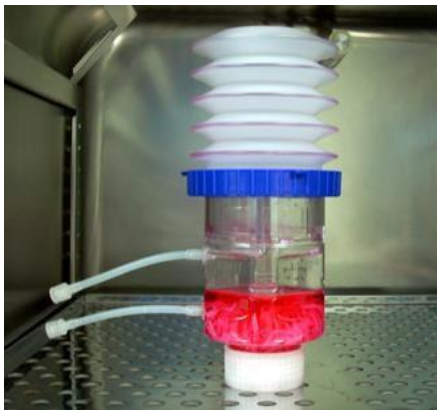
- Add 150 ml culture medium containing cells, viruses, or transfection reagent into the bottle. Cap the bottle with the non-vented cap (white).



- Invert the bottle. Allow the carriers and medium solution drop down to the cap side.

- Tap the bottle and swirl the solution to allow most of the carriers to go down to the cap and be submerged in the medium solution.

- Place the inverted bottle inside an incubator.
- Swirl the bottle every 30 mins for the first hour then every 60 mins for the succeeding hours to evenly mix the solution.



- After incubation is finished, revert the bottle and tap the bottle until most carriers drop down to the matrix basket.
- Add culture medium to make it a total of 500 ml.
- Exchange the non-vented cap with the vented cap (blue) and cap it firmly.
- Place the BelloCell®-500A/AP bottle to BelloStage™ and start culturing according to previous instruction.

Note: The non-vented cap is for single use only and is not autoclavable

5.6.8 Suggested seeding cell amount

Cell strain	Seed quantity (per bottle)
CHO, VERO, BHK, C-127, RK-13, Hela	$0.5\sim1.0\times10^8$
Hybridoma, NS0, Sf-9, Hi-5, Sf-21, HEK-293	$1.5\sim2.0\times10^8$

Stage	Rate of Vertical Oscillation		Holding Time	
	Up	Down	Top	Bottom
Inoculation	2.0 mm/sec	2.0 mm/sec	20 sec	0 sec
Cell Culture	1.0 mm/sec	1.0 mm/sec	10 sec	10 sec

5.6.9 Replenishment of Medium

A general guideline is to replenish media once every day or two, beginning on the third day following inoculation. The frequency depends on residual glucose concentration and pH in the culture media.

- Press "STOP ⃠" to terminate the function of the machine (wait till the internal platform going back to the initial position).
- Remove the BelloCell® bottle from the internal platform of BelloStage™. Wipe the bottle surface with 70% ethanol and then place the bottle in the biosafety cabinet.

Do not spray ethanol on the top of the lid.

- Pre-warm 480 ml of fresh medium at 37 °C.
- Open the lid asceptically; remove the medium by the serological pipette. Add 470 ml of fresh medium (30 ml of conditioned medium usually remains in the bottle). This step should be done as quickly as possible.
- Close the lid, and put BelloCell® bottle back to BelloStage™. Press "START".

Note:

- For the first 2 or 3 days of cell culture, it is not necessary to replace the medium. And then replenish the medium every day.
- It is suggested to determine the time to replace the old medium by detecting the value of pH and glucose concentration in the medium.
- It is allowed to modify the medium pH by modulating CO_2 concentration. Generally speaking, it is necessary to lower down CO_2 concentration when the glucose concentration is sufficient but the medium color changes to orange yellow.

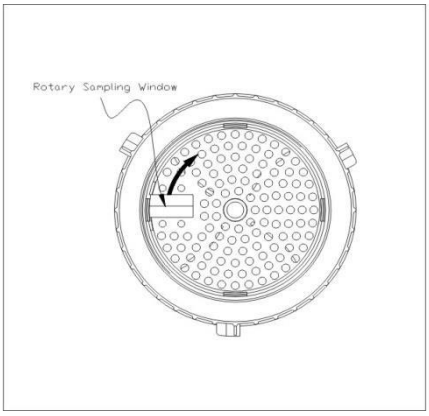
5.6.10 Medium Sampling

- Press "STOP" to terminate the machine, and wait till the internal platform going back to the initial position.
- Wipe the lid and bottle of BelloCell® bottle with 70% ethanol and then place it in the biosafety cabinet. Do not spray ethanol on the lid.
- Open the lid aseptically, and take samples of medium by the serological pipette as quickly as possible.
- Close the lid, and put BelloCell® bottle back to BelloStage™. Press "START".

5.6.11 Cell Observation

During the culture process, it is allowed to sample the matrix BioNOC™ II for cell counting

and observation by a light microscope. In the culture chamber of BelloCell® 500/500P Bottle, there is a sampling window on the rotated partition board, convenient for picking up matrix manually by the forceps.



- Press “STOP ⃠” to terminate the machine, and wait till the internal platform going back to the initial position.
- Wipe the lid of BelloCell® Bottle with 70% ethanol and then place it in the biological safety cabinet. Do not spray ethanol on the lid.
- Open the lid and tilt the bottle. Use the long forceps to penetrate the sampling window and pick up the matrix. Rotate the partition board to pick up other matrices. Close the lid. This step has to be done as quickly as possible.
- Place the matrix on the culture disk.
- Soak the matrix with 99.5% of ethanol for 5 min, dehydrating and fixing the cells on the matrix.
- Wash the matrix with DD water or PBS twice.
- Stain cells by using crystal violet dye, hematoxylin, or Coomassie brilliant blue G stain for 5 to 10 min (follows the standard protocol provided by EBE).
- Wash off the excess dye on the matrix with DD water.

- Crop the matrix appropriately.
- Observe the matrix under a light microscope.

Note: The user should follow local guidelines to handle hazardous waste and biohazardous materials that may be generated from use of this equipment.

5.6.12 Cell Counting (Crystal Violet Nucleus Count)

One method to count cells is to randomly pick six BioNOC™ II or BioMESH™ matrices from the chamber. The quantity of cells on the matrix can be determined by staining of cell nuclei with crystal violet dye.

- Pick up the matrices asceptically by the sterilized forceps.
- Place the matrices in a 1.5 ml eppendorf vial. Each vial contains two pieces of matrices. (triplicate).
- Add 1 ml of CVD in the tube and gently vortex and invert it up and down for 1 min.
- Incubate the tube in the 37°C incubator for at least 1 hours. Gently vortex and invert the tube up and down every 30 min. Count the nuclei of cells in the solution using the hemocytometer.
- Average quantities of cells per matrix, and then multiply the averaged value by 850 (the average number of matrices BioNOC™ II in a BelloCell® Bottle) or 360 (the average number of matrices BioMESH™ in a BelloCell® Bottle) to derive the probable cell population.

Note: This is not an accurate method to assess cell viability.

5.6.13 Cell Count (Trypsinization)

Another method to count cells is to randomly pick six BioNOC™ II or BioMESH™ from the

chamber and then proceed by the following steps.

- Pick up the matrices asceptically by the sterilized forceps.
- Place the matrices in 1.5 ml eppendorf vial. Each vial contains two pieces of matrices (triplicate).
- Add 1 ml of PBS/EDTA (0.02%) to the tube, and wash the matrices by inversion 2~3 times.
- Remove the PBS/EDTA. Repeat the wash at least twice.
- Add 1 ml of trypsin (0.05% trypsin/0.02% EDTA) to the tube. (Trypsin/EDTA concentration may differ depending on existing harvest protocol)
- Incubate at 37°C for 10 min.
- Flick the vial 10 more times by fingers until the solution becomes turbid.
- Count the cells and viability with trypan blue dye exclusive method in the solution using a hemocytometer.
- Average the cell count per matrix, then multiply average value by 850 (the average number of BioNOC™ II disks in a BelloCell® Bottle or 360 (the average number of matrices BioMESH™ in a BelloCell® Bottle) to derive the probable cell population.
-

Note: Cell count might not be accurate if cells cannot release from the matrix efficiently.

5.6.14 Determination of Glucose Concentration in the Medium

During the culture process, it is allowed to monitor the concentration of glucose in the medium to determine the best time to refresh the medium. Meanwhile by deriving the utilization rate of glucose (GUR), the use is able to indirectly understand the growth rate of cells (total quantities of live cells should be proportional to GUR). The calculation formula is

as follows:

$$GUR = \frac{(C_{Glu,t2} - C_{Glu,t1}) \times V_{solu}}{(t_2 - t_1)}$$

GUR	glucose utilization rate [mg/hour]
C_{Glu}	Glucose concentration in the medium [g/L]
T1, T2	Time point to medium sampling [hour]
V_{solu}	Volume of medium [L]

Note:

- It is suggested the glucose concentration at any time point should not be lower than 1,000~1,500 mg/L.
- Please use GlucCell™ glucose monitoring system and GlucCell™ glucose test strip for detecting glucose concentration in the medium.

5.6.15 Cell Harvesting

When the culture process is complete, users may want to harvest cells or cellular products. One solution is to retrieve BioNOC™ II matrices from BelloCell® Bottle then harvest the cells or cellular products either by adding lysis buffer or the freeze-thaw procedure.

5.6.15.1 BelloCell® 500A/500AP Bottle

1. Bring the BelloCell®-500A bottle into a biosafety cabinet, open the cap and carefully discard conditioned culture medium without flushing out the carriers.

2. Add 500 ml pre-warmed HBSS/EDTA (0.5 mM) into BelloCell® bottle and set up the bottle in the BelloStage™. Setting Up/Down speed to 2.0 mm/s; Top/bottom holding time 0 sec. Start BelloStage™ for 5 mins, Stop and discard HBSS/EDTA solutions. Repeat this step twice.

Tips: *This step can wash off trace serum and non-viable cells. Some cells will be dislodged during this step; most of them should be nonviable cells. This step can increase the harvest viability by removing non-viable cells first. After the second run of HBSS-EDTA wash, cell collection may be started if viability is acceptable.*

3. Add 110 ml pre-warmed Trypsin solution (0.05%/0.5 mM EDTA), Cap with the non-vented cap (white) firmly. Invert the bottle and tap the bottle until all carriers drop down to the cap side and are submerged.

4. Incubate the bottle in a CO_2 incubator for 10 mins. Revert the bottle back to an upright position and tap the bottle until all carriers drop back to the matrix basket. Allow trypsin flow down to the bellows. Incubate for additional 10~20 mins.

Tips: *sufficient time for enzymatic digestion is critical for a successful cell harvest. Most cells can withstand trypsin-EDTA for more than 30 mins without altering viability. High cell density will require more trypsin and time to digest.*

5. Tap the bottle sharply and steadily against your palm in the upright position for 3 mins. Rotate the bottle during tapping.

6. Add 5 ml 0.5 mg/ml DNase (optional) and 25 ml 0.1% trypsin inhibitor (or serum) into 150 ml culture medium, or HBSS. Add the 150 ml solution into the bottle. Cap the non-vented cap firmly and invert the bottle. Swirl to wash off the cells from the carriers.

7. Pour the cell-laden solution into centrifuge bottles. Place the centrifuge bottles into centrifuge. Harvest cells by centrifugation.

8. Tap the bottle as in step 5. Pour the supernatant from the centrifuge bottles back to the BelloCell® bottle and wash off cells from the bottle. Repeat step 5 to 7 two more times until cell residues below an acceptable range (take carrier samples to check the harvest efficiency). Collect all cell pellet together. Check the cell density and viability.

Note: The non-vented cap is for single use only and is not autoclavable.

5.6.15.2 BelloCell® 500/500P bottle

1. For cellular product, decant all the medium in BelloCell® bottle.

2. Add 500 ml pre-warmed PBS/EDTA into the bottle. Start BelloStage™ and apply parameters Up rate: 2.0 mm/sec; Down rate: 2.0 mm/.sec for 5 min.
3. Stop BelloStage™ and decant the solution.
4. Repeat steps 2 and 3 at least twice.
5. Add 400 ml of pre-warmed trypsin/EDTA solution (0.05%/0.5 mM) to BelloCell® Bottle. Start BelloStage™ and apply parameters Up rate: 2.0 mm/sec; Down rate: 2.0 mm/.sec for 10~15 min.
6. Stop BelloStage™. Aseptically discard the enzymatic solution, and incubate for 10~15 mins more.
7. Tap the bottle sharply against your hand or a soft, round counter edge, at the matrix chamber level, for 3 ~5 min, to force cells to release from the carriers and the matrices they have created.
8. Add 500 ml Culture medium or PBS containing FBS or trypsin inhibitor to BelloCell® bottle. Start BelloStage™ and apply parameters Up rate: 2.0 mm/sec; Down rate:
9. 2.0 mm/.sec for 2 to 3 min.
10. Stop BelloStage™. Move BelloCell® Bottle to the biosafety cabinet, aseptically harvest the cell-laden solution, and then harvest the cells by centrifuging the solution.
11. Repeat above 3 steps to harvest cells as much as possible.

Note: Because of the high cell density, cell-laden solution may contain many extra-cellular matrices. But it can be separated from cells by centrifugation.

Chapter 6 Maintenance

6.1 Cleaning

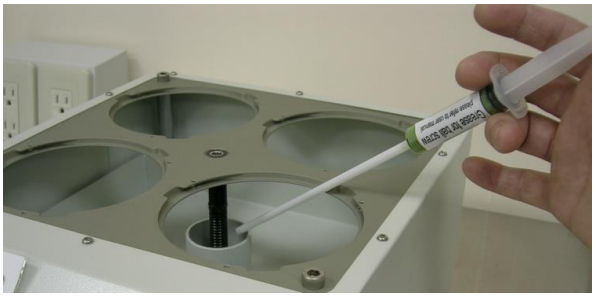
Periodic maintenance is essential to the proper operation of your BelloStage™ system. Periodically clean the BelloStage™ main body and control panel by wiping the outside surfaces with a soft cloth with a 70% alcohol solution.

Note: The exterior surfaces of BelloStage™ main body and the control panel are coated with an anti-corrosion material. Never use any abrasive materials or tools and be careful not to scratch the coating.

6.2 Maintenance

It is recommended to grease the BelloStage™ platform ball screw once every three months, to keep it operating smoothly:

- Turn off and unplug the BelloStage™.
- Remove the main body from your incubator.
- Slowly and carefully apply approximately 0.5 ml of grease, using a user-supplied syringe, to the platform ball screw, from the bottom up.
- The maintenance record can be recorded in the maintenance card.



Chapter 7 Troubleshooting

Index	Possible Cause	Solutions
Continuous alarm, internal platform fail to move in the unit	1. Rust on the ball screw cause resistance to the platform movement 2. Malfunction of the step motor 3. Loose signal cable between control panel and the main body.	1. Remove the rust and grease the ball screw. Be sure to grease the ball screw every 3 months. Remove the BelloStage™ main body from the incubator if not use for a while. 2. Replace the step motor. 3. Make sure the 9-pin signal cable between control panel and platform unit is well connected.
The control panel looks normal, but the internal platform ceases movement	1. The 6V DC-DC circuit is defective. 2. Malfunction of the step motor.	1. Replace the 6V DC-CD circuit. 2. Replace the step motor.
Random codes on the display, but unit operates normal	1. Signal transfer between LCM and CPU abnormal. 2. Noise from other electrical signal.	1. Reset and restart. 2. Try to use an independent power source of the equipment.
Control panel cannot be attached on the incubator.	1. Dirt between the magnetic patch and the incubator. 2. Wall surface of the incubator is not flat. 3. Wall surface is inert to magnetic.	1. Clean up the dirt. 2. Find a smooth and flat surface. 3. Find a surface made by iron or steel.
Keypad failure	Keypad failure	Change keypad

Chapter 8 Related Products

Item	Product Name	Item Code
1.	BelloCell®-500A BioNOC II™ 100 ml (BioNOC™ II 5.5 g), pack of 4	1400003
2.	BelloCell®-500AH BioNOC™ II 50 ml (BioNOC™ II 2.8 g), pack of 4	1400297
3.	BelloCell®-500AQ BioNOC™ II 25 ml (BioNOC™ II 1.4 g), pack of 4	1400298
4.	BelloCell®-500AP BioNOC™ II 100 ml (BioNOC™ II 5.5 g), pack of 4	1400004
5.	BelloCell®-500APH BioNOC™ II 50 ml (BioNOC™ II 2.8 g), pack of 4	1400301
6.	BelloCell®-500APQ BioNOC™ II 25 ml (BioNOC™ II 1.4 g), pack of 4	1400302
7.	BelloCell®-500A BioMESH™100 ml (BioMESH™ 18 g), pack of 4	1400295
8.	BelloCell®-500AP BioMESH™100 ml (BioMESH™ 18 g), pack of 4	1400303
9.	BelloStage™ Set Including: 1. BelloStage™ Console x1 pc 2. Control panel x1 pc 3. 100~240V power adapter x1 pc 4. Signal cablex1 pc 5. Forceps x 2 pc 6. CVD kit (50 ml) x 1 bt 7. Ball Screw Grease(SR-1)x 5 ml/bt	2230005
10.	BelloCell® System Complete Set Including: 1. BelloStage™ Set x1 set 2. GlucCell Glucose Monitoring System x 1 set	2230006

11.	BelloCell® Continuous System Complete Set Including: 1. BelloStage™ Set x1 set 2. BelloFeeder Pump Set x1 set 3. Tubing Complete Set x2 sets 4. GlucCell Glucose Monitoring System x1 set	2230007
12.	BelloFeeder™ Pump Set, 1pc/set	1400067
13.	GlucCell™ Glucose Monitoring System Including 1. GlucCell™ Meter x1 pc 2. Glucose Test Strip (25 pc x 2 bt) x1 pc 3. Check Card x1 pc 4. Bag x1 pc	1400009
14.	GlucCell™ Glucose Test Strip, 25 pc/bt, 2 bt/bx	1400010
15.	Tubing Complete Set Including: 1. Tubing Set x1 set 2. PP Pump Head x1 pc 3. Head Plate * 1 pc	1400011
16.	Disposable Silicone Tubing Set & Pump Head Including: 1. Tubing Set x1 set 2. PP Pump Head x1 set	1400012
17.	Disposable Silicone Tubing Accessory, 5 pcs/bx	1400013
18.	Crystal Violet Dye Nucleus Count Kit, 100 ml/bt	1400014
19.	Filtered Cap, pack of 6	1400015
20.	Non-vented Cap, pack of 8	1400016
21.	Forceps , 25 cm, 1 pc	1400017

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