CESCO®

BelloCell®

High-Density, Disposable Cell Culture System

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Disclaimer Notice

CESCO Bioengineering Co., Ltd. reserves the right to change information in this document without notice. Updates to information in this document reflect our commitment to continuing product development and improvement.

Manual Conventions

Bold	**Text in bold face type emphasizes key words or phrases.**
NOTE:	**Notes contain essential information that deserves special attention.**
CAUTION!	***Caution* messages appear before procedures which, if caution is not observed, could result in damage to the equipment.**
WARNING!	***Warning* messages alert you to specific procedures or practices which, if not followed correctly, could result in serious personal injury.**
BIOHAZARD WARNING!	***Biohazard Warnings* alert you to user responsibilities in the handling and disposal of potentially hazardous biological material.**

Warranty

CESCO Bioengineering Co., Ltd. warrants that if a product manufactured by CESCO 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 CESCO directly or (ii) the date the product is purchased from authorized distributors by CESCO (the “Commencement date”). Except as expressly stated above, CESCO 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 CESCO must perform all warranty inspections. In the event of defect covered by CESCO’s warranty, CESCO 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-CESCO’s authorized spare parts, illegal disassembly, etc., relocation or deterioration caused by application which is not the original purpose to design this product.

CAUTION!

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.

CESCO Bioengineering Co., Ltd. (CESCO) is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by CESCO.

1 SAFETY INFORMATION

- Please read this entire instruction manual prior to use the system.
- The BelloStage®-3000 system operates on 100 – 230 volts, 50/60 Hz through a desktop AC transformer. **Note:** Ensure that the input voltage listed on the AC Transformer label is compatible with your line voltage.

> ***NOTE***
> Ensure that the input voltage listed on the AC Transformer label is compatible with your line voltage

- Use a properly grounded electrical outlet of correct voltage and current handling capacity.
- Do not disassemble the BelloStage®-3000 or AC Transformer.
- Do not submerge either the BelloStage®-3000 system or BelloCell® in water.
- Do not place the control box inside the incubator. The control box is designed to operate outside the incubator.
- The BelloCell ® culture system is for laboratory use only. It is not intended for diagnostic or therapeutic use in humans or animals.
- One BelloCell® is one-time use only. When the experiments are finished, BelloCell® should be decontaminated and discarded. Consult local institution for detail.

> WARNING ***WARNING!***
> **Avoid inserting your hands inside the BelloStage® unit during operation.**

- Please feel free to contact CESCO Bioengineering Co., Ltd. at info@cescobio.com.tw for technical assistance.

2 PRODUCT SPECIFICATION

* BelloStage®-3000 Console:

Dimension	264 x 359 x 170 m/m W/L/H (10.4 x14 x6.7 inches)
Weight	7.0 kg (15.4 lb)
Power	100~230 VOLTS AC, 50/60 Hz (Input); 12 VOLTS DC (Output)
Up-Down Rate	0.25 to 2.0 mm/sec ; Step of 0.25 mm/sec
Delay Time	0 to 99 min 59 sec. Step of 1.0 sec or 1.0 min.
Driver Motor	DC stepping-motor
Environment	20 ~ 42 ℃, 0 ~ 90% relative humidity (in a CO_2 incubator)
Mechanical Protection	Hi-Low Optical Sensor
Transmission	Gear set (ratio 1:1.2) and belt
Materials	Aluminum alloy, chromic steel

* BelloStage®-3000 Control Box:

Dimension	137 x 226 x 40 m/m L/W/D (5.4 x 8.9 x 1.6 inches)
Weight	1.16 kg (2.6 lb)
Power	100~230 VOLTS AC, 50/60 Hz (Input) ; 12 VOLTS DC (Output)
Environment	Room temperature (outside CO_2 incubator)
Materials	Magnetic backplate, to hold the controller to the side of the incubator

* BelloFeeder®-1300 Pump:

Dimension	131 x 230 x 61 m/m W/L/H (5.2 x9.1 x2.4 inches)
Weight	0.73 kg (1.6 lb)
Power	85~250 VOLTS AC, 50/60 Hz (Input); ~5 A
Environment	20 ~ 42 ℃, 0 ~ 85% relative humidity (in a CO_2 incubator)

* BelloCell®-500 Bottle:

Dimension	H 243 mm × Diameter Ø 100 mm
Working Volume	500 ml
Carriers Volume	100 ml, 5.5 g
Vent Filter type	0.22 μm PTFE membrane with PP support
Carrier type	**BioNOC II®** carriers
Material	PETG, LDPE/EVA and PP

* BelloCell®-500P Bottle:

Dimension	H 243 mm × Diameter Ø 100 mm
Working Volume	500 ml
Carriers Volume	100 ml, 5.5 g
Vent Filter type	0.22 μm PTFE membrane with PP support
Carrier type	**BioNOC II®** carriers

Material	PETG, LDPE/EVA and PP
Inward-flowing port	10 cm silicon tubing with PP female luer connector
Outward-flowing port	10 cm silicon tubing with PP male luer connector

* BelloCell System Part Numbers:

Item	Part number
BelloStage system	BS3000
BelloFeeder-1300	BF1300
BelloCell-500 bottle	BC0500
BelloCell-500E bottle	BE0500
BelloCell-500H bottle	BH0500
BelloCell-500P bottle	BC1000
BelloCell-500AP bottle	BA1000
BelloCell-500EP bottle	BE1000

3 SYSTEM DESCRIPTION

BelloCell® High-Density Cell Culture System provides a protected, controlled, and contained environment for the growth of cell cultures. The **BelloCell®** High-Density Cell Culture System consists of three major components (see *Figure 1*): a control box, the **BelloStage®** unit, and ready-to-use disposable 500 ml bottles. The unit fits easily inside your incubator, while the magnetic control box attaches conveniently to an outside metal incubator wall for easy access.

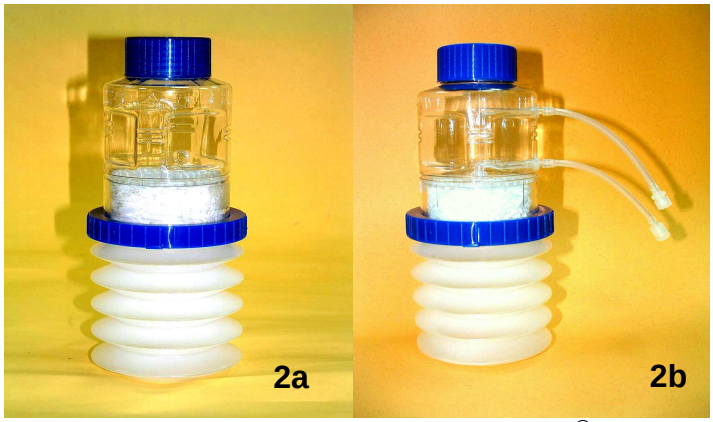
Figure 1. BelloCell Cell Culture System

Control box

BelloStage® Unit

The BelloStage® unit, which holds up to four disposable cell culture bottles, moves the bottles' contents up and down according to your program, using a platform to compress and expand the bellows built into each bottle, to optimize oxygenation. As the platform lifts, it compresses the bellows, sending the media into the chamber that contains the **BioNOC II®** disks; as the platform descends, the media returns to the expanding bellows, exposing the carrier disks to the atmospheric environment.

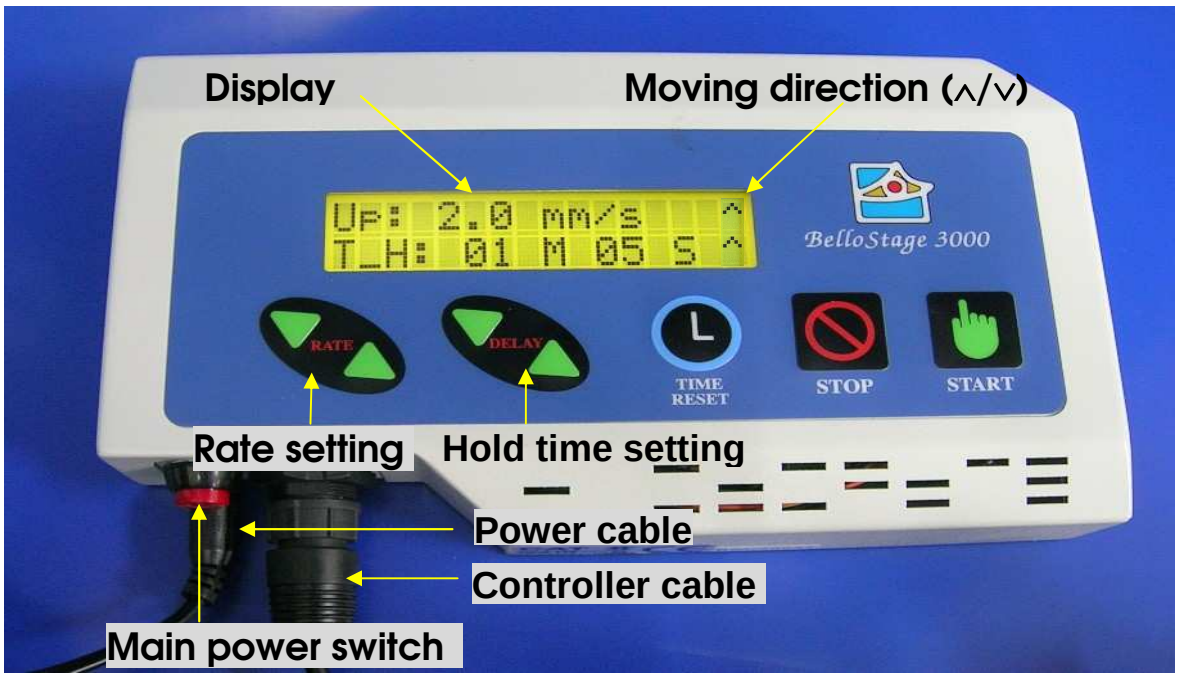
Figure 2. BelloCell-500 bottle (2a) and BelloCell-500P bottle (2b)



BelloCell®-500 (see *Figure 2a*) is made of disposable plastic materials. **BioNOC II®** carriers are packed in the upper chamber of a BelloCell®-500 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®-3000. **BelloCell®-500P** (see *Figure 2b*) not only has the same features of BelloCell®-500 but can perform more advanced perfusion/re-circulation operation through its inlet and outlet ports to stabilize culture environment and reduce labor efforts.

4 CONTROL BOX FEATURES

Figure 3. BelloStage Control Box



* Button Functional Description :

To start operation

1. Stop operation

2. Switch the setup mode between “Up/T_H” and “Down/B_H” (T_H: top holding time; B_H: bottom holding time)

Alert mode / Culture time recording/View the BelloStage version

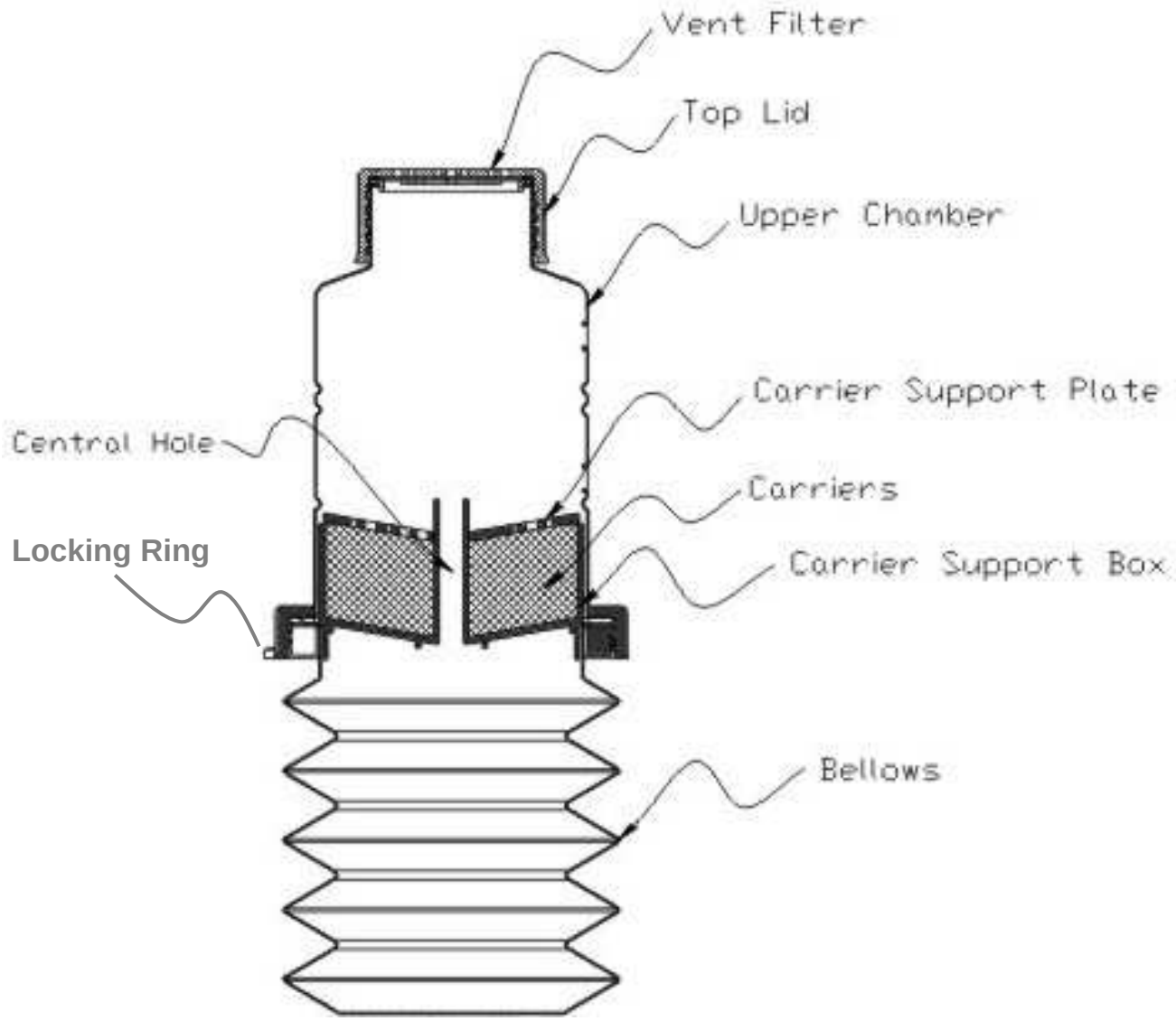
Setup top (T_H) and bottom holding time (B_H) (0~99 mins 59 secs) before or during operation

Setup up and down speed (0.25 to 2.0 mm/s) before or during operation

5 BelloCell®-500 BOTTLE FEATURES

The following corss-section of the BelloCell® bottle (see *Figure 4*) will acquaint you with its features and components:

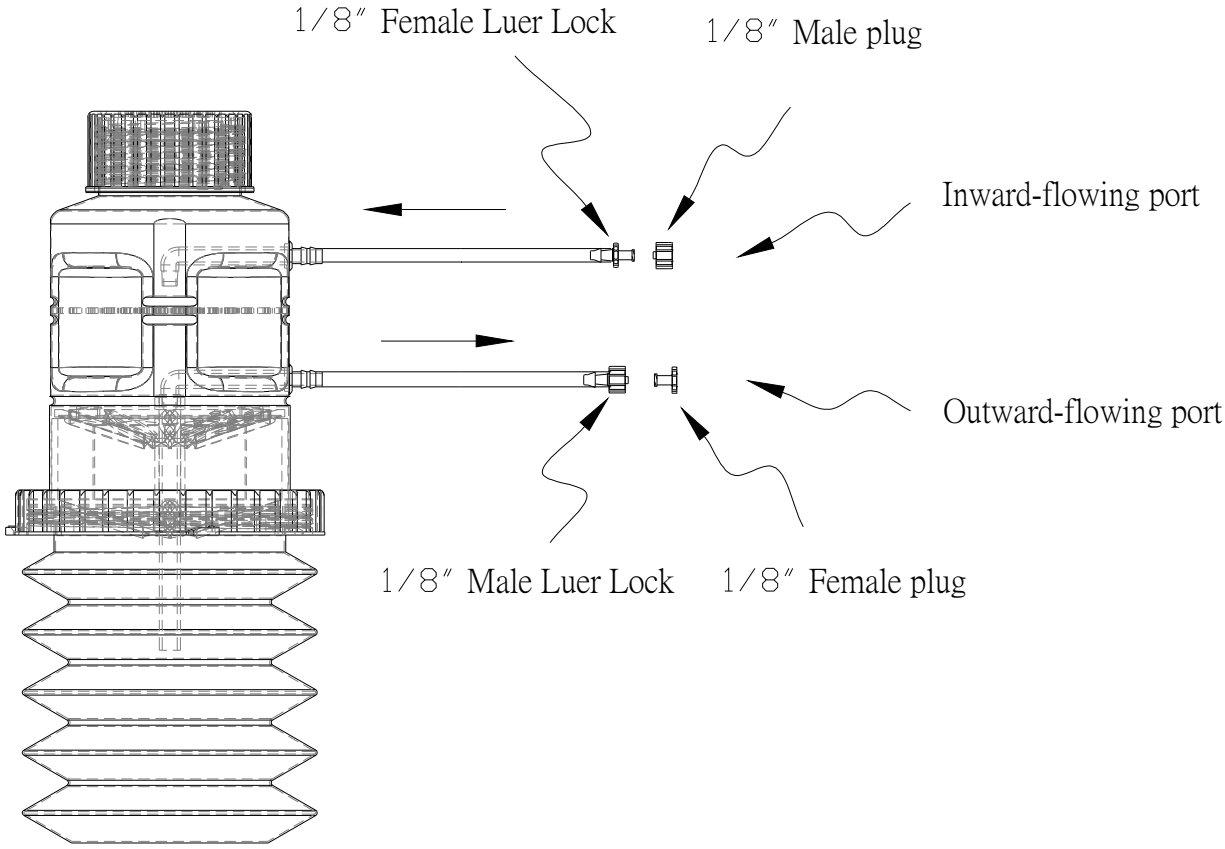
Figure 4. BelloCell Bottle Corss-Section



6 BelloCell®-500P BOTTLE FEATURES

The cross-section of a BelloCell®-500P bottle (see *Figure 5*) will acquaint you with its features and components. Basic parts of BelloCell®-500P are the same with those in BelloCell®-500 besides inward-flowing and outward-flowing connectors.

Figure 5. BelloCell-500P Bottle Perspective Drawing



7 BelloCell®-500 & BelloStage®-3000 DIMENSIONS

Figure 6. BelloCell® Bottle Dimension

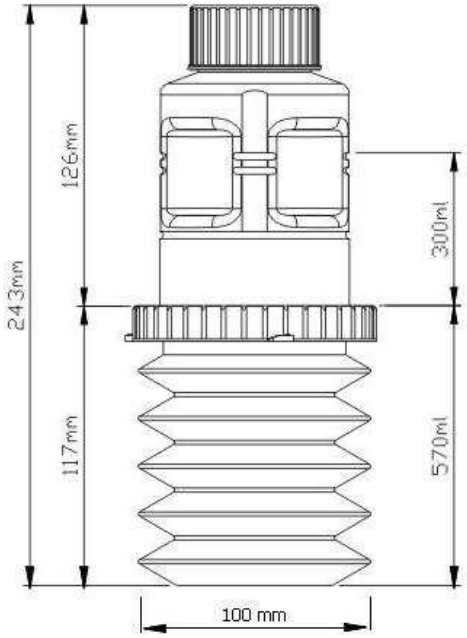
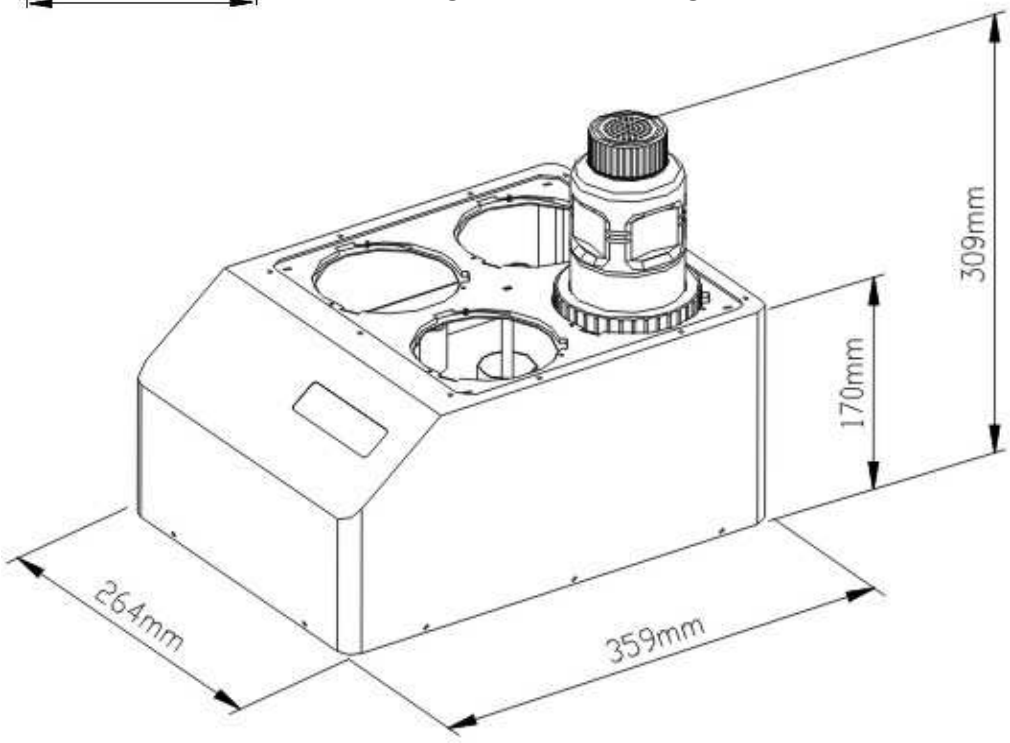


Figure 7. BelloStage® Dimension



8 UNPACKING & INSTALLATION

8.1 Unpacking

After you have received your order, inspect the boxes carefully for any damage that may have occurred during shipping. Report any damage, missing pieces immediately to the carrier and to your local CESCO distributor. Save all packing materials and this User's Guide.

8.2 Packing List Verification

Verify on your packing list that you have received the correct materials. Report any errors to the carrier or to your local CESCO distributor.

8.3 Environment

The BelloStage® unit is designed to operate optimally in the following ambient conditions:

- 20º to 42℃
- 0 to 90% Relative Humidity (inside a CO_2 incubator)

8.4 Electrical Requirements

With the AC transformer provided, the BelloStage® system 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.

CAUTION!

A grounded electrical outlet is necessary for the safe operation of this instrument.

8.5 Installation (see *Figure 8*)

1. Connect the controller signal cable on the left side of BelloStage® unit to the control box at the 9-pin cable junction.

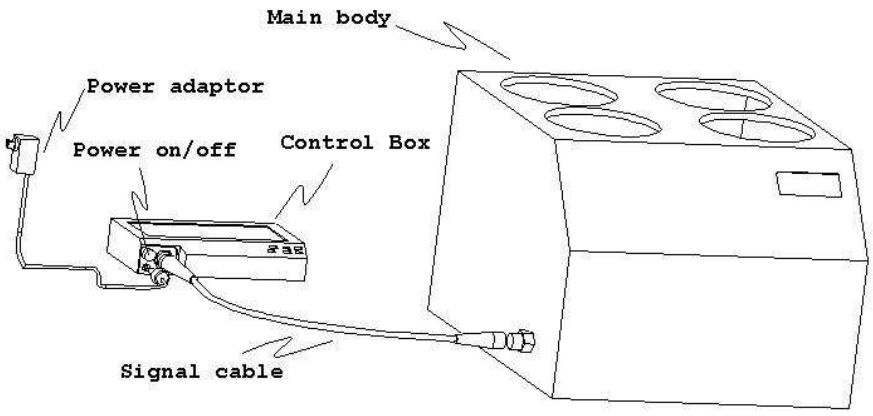
2. Place the BelloStage® platform inside a humidified CO_2 incubator, and leave the control box outside the incubator.

3. Thread the controller signal cable through an available port or between the door and door gasket..

4. The control box is magnetized for easy attachment. Position the control box on a convenient, metallic, outside wall of the incubator.

5. After verifying that your unit's AC transformer plug mates to your outlet, connect the power cable from the control box to your power supply outlet. The BelloStage® platform is ready to use now.

Figure 8. BelloStage Assembly



CAUTION!

Always turn off, unplug and remove the BelloStage® unit from the incubator if you do not plan to use the system for a while.

9 IMPORTANT CAUTIONS & WARNINGS

WARNING!

Never place your hands inside the BelloStage® unit during operation.

CAUTION!

Never spray alcohol on the BelloCell® bottle cap because it contains a sterile filter.

CAUTION!

Never flame the BelloCell® bottle!

CAUTION!

A grounded electrical outlet is necessary for the safe operation of this instrument.

CAUTION!

The exterior surfaces of the BelloStage® unit and controller are coated with an anti-rust material. Never use any abrasive materials and be careful not to scratch the exterior finish.

BIOHAZARD WARNING!

The user is responsible for following local guidelines for handling hazardous waste and biohazardous materials that may be generated from the use of this equipment.

10 OPERATION

10.1 General Instruction of BelloStage®:

Take a moment to acquaint yourself with the use of the controller keypad, to set up operating parameters before operation begins and to see how to change operating parameters during a run.

10.1.1. Before operation:

(1). Press the **STOP** key to switch between the “Up/T_H” and “Down/B_H” in the display, whre **T_H** means **Top Holding time** and **B_H** means **Bottom Holding time** (see *Figures 9 & 10*).

Figure 9: Main Screen—Rising Platform

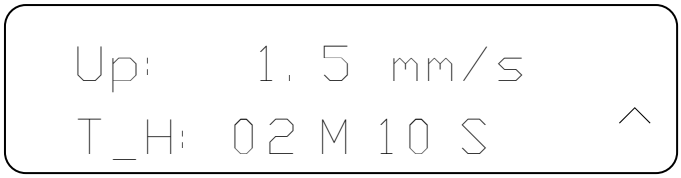


Figure 10: Main Screen—Descending Platform

(2). Press the ”▲”or ”▼” part of the **RATE** key to change platform rising and lowering speed. Eight moving speed are available, in increments of 0.25, from 0.25 to 2.0 mm/s

(3). Press the ”▲”or ”▼” part of the **DELAY** key to change the platform holding time at the top (compressed bellows) and bottom (extended bellows) positions. Holding time can be set from 0 to 99 minutes and 59 seconds. To set minutes, press and hold either DELAY arrow key; to set seconds, press either DELAY

arrow key in short bursts.

(4). Start and stop

Press **START** to begin culture process. Press **STOP** to terminate culture process.

(5). Press the **TIME RESET** key to change the display between “Total Time”, “Alert Mode” and the current BelloStage system version.

- In the “**Total Time**” display, press the “Time Reset” key and hold it down for 3 seconds to reset the time record to zero.
- In the “**Alert Mode**” display, press the ”▲”or ”▼” part of the DELAY key to change alarm mode.

- In “30 seconds” mode, alarm will keep beeping for 30 seconds.

- In “**Continuous**” mode, the alarm will keep beeping until the “STOP” key is pressed.

10.1.2. During operation

- To change the platform rising and lowering speed, press the ▲ or ▼ part of the **RATE** key. As the platform is rising, you can change the rising rate; and as the platform is descending, you can change the lowering rate.
- To change the platform holding time at the top (compressed bellows) and bottom (extended bellows) positions, press the ▲ or ▼ part of the **DELAY** key. As the platform is rising, you can change the upper holding time (T_H); and as the platform is descending, you can change the lower holding time (B_H). While the platform is holding, you cannot change the hold time.
- Press the **TIME RESET** key to check the culture time in the Total Time or the Alert Mode display, but you cannot change the values during operation.
- Press the **STOP** key to stop platform movement; the platform will by

default return to its lowest position, leaving the bottle bellows extended.

10.1.3 Stopping a Run

At any time during a run, you can stop the BelloStage unit by pressing the **STOP** key on the controller. The moving plate will go down to the bottom position. A "**Return to Base Waiting…**" will be displayed in the LCD screen (see *Figure 11*). Two "BEEP" sound appeared indicate the termination of the operation.

Figure 11. Display after pressing STOP key

10.2 Running a Culture

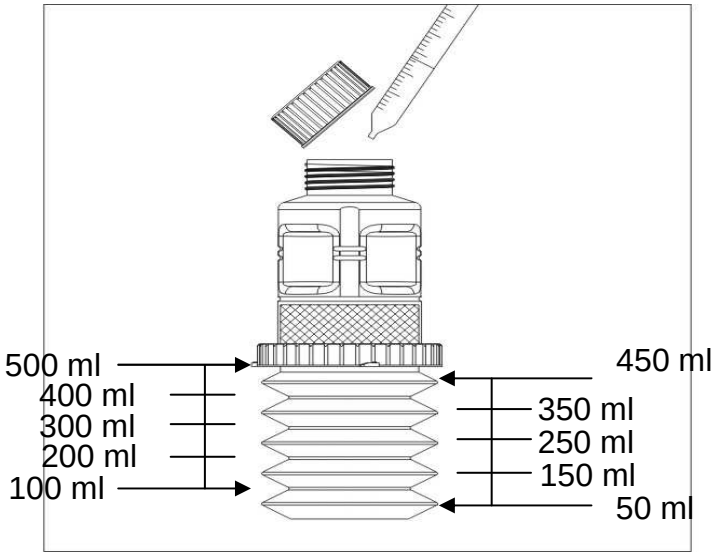
10.2.1 Running a Culture in **BelloCell®-500**

CAUTION!

- **Be sure to read Sections 10 before taking any action to prepare for or to run a culture.**
- **Never spray alcohol on the BelloCell® bottle cap because it contains a sterile filter.**
- **Never flame the neck of the BelloCell® bottle.**

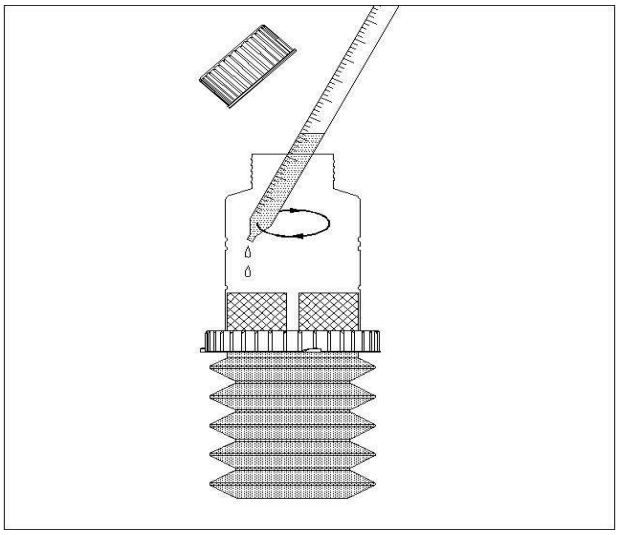
Step 1. Remove a fresh BelloCell®-500 or BelloCell®-500P bottle from its bag. It is already sterilized for use. Place the BelloCell® unit in a bio-safety cabinet. Aseptically remove the cap and fill in 470 ml pre-warmed culture medium**.** Tilt the bottle to send all the media down into the bellows chamber.

Figure 12. Adding Media to BelloCell® Bottle



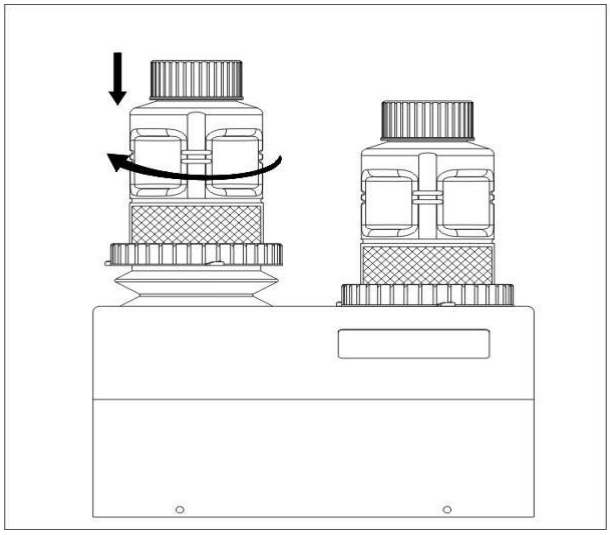
Step 2. Aseptically open the cap, and – directly on top of the BioNOC® II disks, working in a spiral from the center- dispense 30 ml cell suspension containing a total of **1 x 10^8 ~ 2.0 x 10^8 live cells.** Close and tighten the cap.

Figure 13. Inoculation to BelloCell® Bottle



Step 3. Bring the bottle to **BelloStage®** unit in the incubator, making sure the blue ring locks in place. Turn on the controller by pressing the red ON/OFF button. Set up the moving rate and holding time parameters for immobilization. Push " START " button to progress inoculation procedure.

Figure 14. Loading BelloCell® Bottle in Unit



NOTE

Do not delay: start operation immediately after inoculation.

NOTE

pH is critical at this point. After inoculation, make sure that any added media has a pH of 7.0-7.2 if the media contains sodium bicarbonate. CO_2 concentration should be maintained between 5-10% in the incubator. Abnormal pH range will retard the immobilization efficiency.

Step 4. After 2~5 hours, reset the moving rate (Rate) and holding time (T_H, B_H) parameters for culture.

NOTE

Increase top-holding time allows more nutrient exchange between bulk medium solution and medium carriers. Increase top-holding time can also reduce oxygen level if the condition prefers low dissolved oxygen.

Increase bottom-holding time will increase the oxygen level during culture. Increase bottom-holding time can also help to stabilize the pH.

Recommended inoculation densities per bottle

Cell Type	Inoculation density (cells/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	$1.5\sim2.0\times10^8$

Recommended Operating Parameters

Procedure	Rate(mm/sec)		Delay	
	UP	DOWN	T_H	B_H
Inoculation*	2.0	2.0	20 secs	0
Cell Culture	1.0~1.5	1.0~1.5	10 secs	1 min~30 mins

*for the first 2~5 hours following inoculation

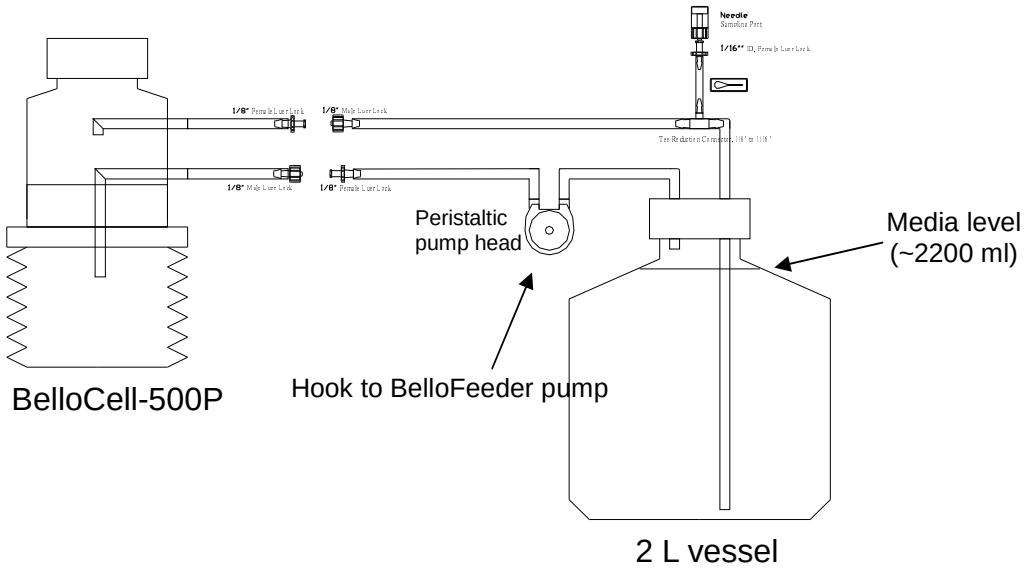
10.2.2 Running a Culture in **BelloCell®-500P**

> ***NOTE***
>
> Cell inoculation procedure is the same as Section 10.2.1 BelloCell®-500

> ***NOTE***
>
> Sterilize the tubing set and reservoir vessel by autoclaving at 121℃, 30 mins prior for use.

> ***NOTE***
>
> Connect the **tubing set** with BelloCell-500P bottle and start pumping after 48~72 hours culture according to the *figure 15* shown below.

Figure 15. BelloCell-500P Bottle Assembly



1. Prior to connect to BelloCell-500P bottle, fill the culture medium into the reservoir vessel and remain the headspace in the reservoir to be as little as possible. (Large headspace (> 500 ml) will cause variation of the medium level in BelloCell)

CAUTION!

No matter what's the volume of the vessel, keep the headspace in the vessel below 500 ml after fill with culture medium.

2. Make sure the cap in the reservoir vessel is firmly capped and air tight.

CAUTION!

Any leakage from the cap will cause contamination and break the balance during medium recirculation.

3. Make the connection as the suggested drawing
4. Plug the pump head onto **BelloFeeder pump**. (please refer to BelloFeeder-1300 Pump Quick Reference Sheet)
5. Initial pumping rate should be around 500 ml to 1000 ml per day, and gradually increases to 1999 ml/day according to the glucose level.
6. Take samples through the sample ports by syringe.

10.2.3 Sampling Media for BelloCell-500

1. To take a sample, if one is needed, press the **STOP** key to stop the platform and, by default, send it back to its lowest position.
2. Unlock the BelloCell® bottle and remove it from the unit.
3. Wipe the BelloCell® bottle with a 70% alcohol solution, but **do not spray alcohol on the cap** because it contains a filter.
4. Aseptically open the cap, tilt the bottle slightly and pipette out the desired amount of sample through the central hole in the BioNOC® II chamber (*see Figure 4*).
5. Close the cap tightly and reinstall the bottle in the BelloStage® unit, locking it in place, then press the **START** key again.

10.2.4 Sampling Media For BelloCell®-500P

1. Use sterilized syringe and withdraw samples through silicon plug of the sampling port. Withdraw 1.0 ml medium or more for analysis.

> ***NOTE***
>
> ***Block the sampling tubing first by slide clamps before draw out needle to avoid back-flow of the culture medium.***.

10.2.5 Adjusting pH Level

The CO_2 concentration in the incubator may have to be adjusted manually to control the pH level of the BelloCell® bottle's contents. A good rule of thumb is that while the glucose concentration in the bottle is still sufficient and the media color has turned orange-yellow, it is time to adjust the CO_2 concentration to a lower percentage.

10.2.6 Media Replenishment For BelloCell®-500

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.

Whenever there is a need to change the culture media, follow the procedure in Section 10.2.4 above for aseptically taking a sample, but use the pipette to remove *all* of the media. Then add **470 ml** of fresh media (30 ml of conditioned media usually remains in the bottle). Return the bottle to the BelloStage® unit and press the **START** key to resume the run.

10.2.7 Media Replenishment For BelloCell®-500P

1. Turn off **BelloFeeder® pump** and BelloStage®-3000.

3. Block medium flow by clamps in both in- and out-flow tubings. Separate pump head from BelloFeeder® pump module (please check Step-by-Step Instruction for details). Move and place the BelloCell-500P, medium reservoir and the tubing set inside a biosafety cabinet.

4. Prepare a reservoir containing fresh culture medium. Open and move the cap set from the conditioned to the new reservoir and close it *tight*.

5. Place the BelloCell®-500P, pump head and medium reservoir together inside the incubator again and insert the pump head into the rotary shaft of BelloFeeder® pump. Loosen the clamps on the tubing. Turn on the BelloStage®-3000 and BelloFeeder® pump.

WARNING!

Before turn on BelloFeeder pump module, both the slide clamps on the in and out-flow tubing must be loosened !!

10.2.8 Monitoring Glucose*

You can measure the glucose residue in a sample of the media from time to time to determine whether the media should be renewed. You can also calculate the Glucose Uptake Rate (GUR) to monitor the cell growth rate; the GUR value should be proportional to the total cell volume. To calculate GUR, use this equation:

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

where GUR is the Glucose Uptake Rate in mg/hour
C_{Glu} is the concentration of residual glucose
t1 & t2 are the sampling hours
V_{solu} is the volume of media in liters

* Cesco Bioengineering also provides **GlucCell® glucose meter** and kits for your convenience to measure glucose concentration during culture. Please refer to Section 15. Options for BelloCell® Cell Culture System.

NOTE

We recommend that you maintain the glucose concentration at more than 1000 mg/L.

10.3 Cell Harvest and Sampling

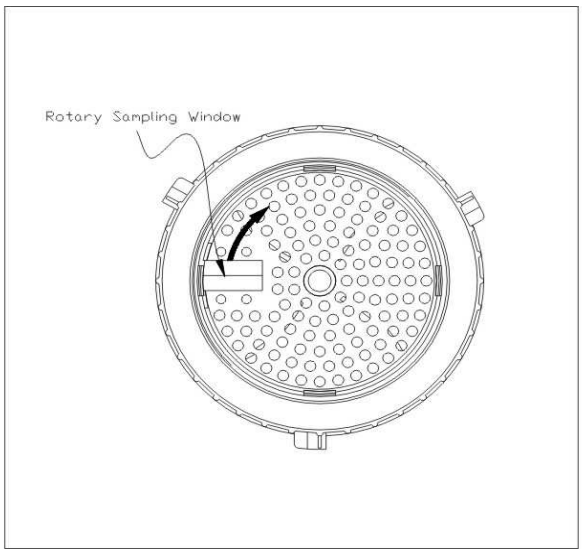
10.3.1 Sampling of BioNOC II Carriers

The BelloCell® bottle is equipped with a sampling port (*see Figure 16*). The rotating BelloCell® chamber can be turned to allow sampling of carriers anyplace in the chamber.

To sample BioNOC® II disks:

1. Press the **STOP** key on the controller to return the platform to its bottom position and to halt operation.
2. Unlock the BelloCell® bottle from the unit and aseptically open the cap.
3. Tilt the bottle toward you until you can see the sampling window (*see Figure 16*).
4. Aseptically push against the sampling window with the tip of a sterilized forceps, then pick the carriers.
5. Place the selected carriers in a sterilized vessel.
6. Use the tip of the same forceps to rotate the sampling window as necessary to obtain all the desired carriers.

Figure 16. BelloCell® Sampling Window
(view from top of BelloCell® bottle, cap removed)



10.3.2 Observing Cells

To observe a sample of cells under a light microscope:

1. Aseptically remove a few BioNOC® II disks from the carrier disk chamber, following the aseptic instructions in Section 10.3.1 above.
2. Dehydrate and fix the cells in their carriers using EtOH 70% dehydration or 99.5% EtOH dehydration for 5 minutes.
3. Wash off the EtOH twice, using either DI water or PBS.

4. Stain the cells with hematoxylin, crystal violet or coomassie brilliant blue G dye for 5-10 minutes.
5. Wash off the excess dye with DI water.
6. Observe the cells in your light microscope (*see Figures 17a and 17b*).

BIOHAZARD WARNING!
The user is responsible for following local guidelines for handling hazardous waste and biohazardous materials that may be generated from the use of this equipment.

Figure 17a. Hi-5 Cells in BioNOC II disks (Stained with hematoxylin)

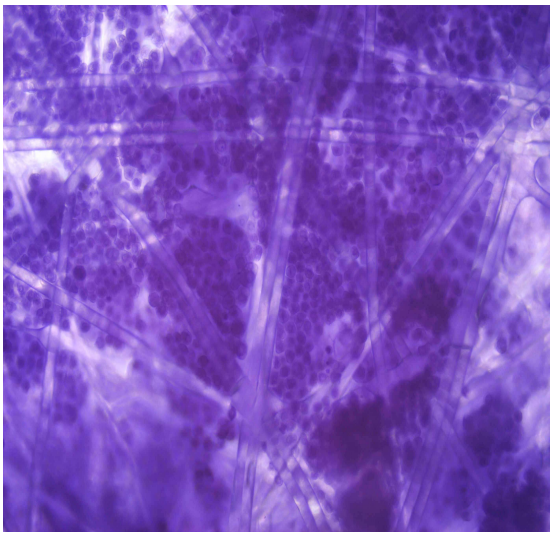
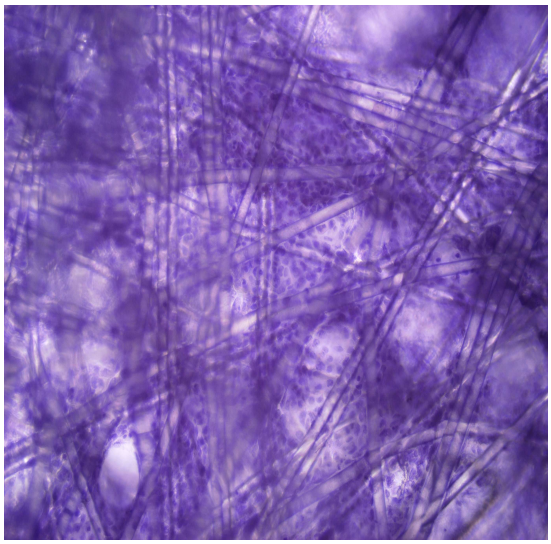


Figure 17b. HEK293 Cells in BioNOC II disks (Stained with hematoxylin)



10.3.3 Counting Cells

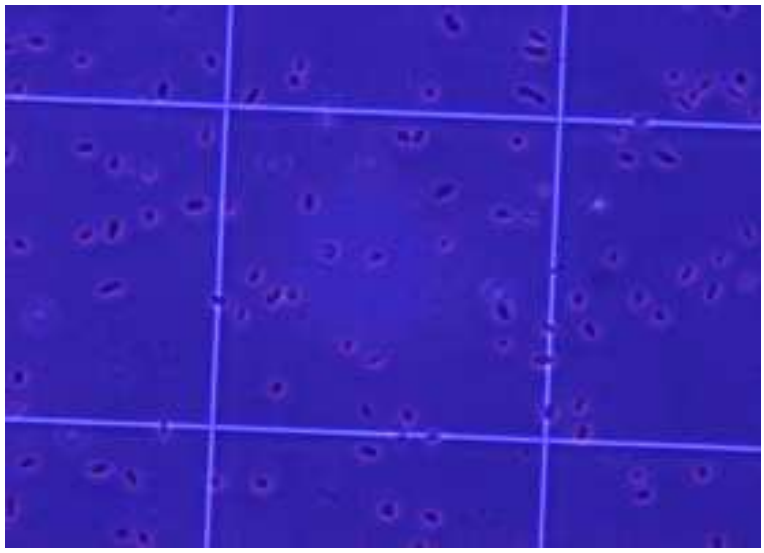
10.3.3.1 Crystal Violet Nucleus Count Method

One method to count cells is to randomly pick six BioNOC® II disks from the carrier disk chamber, following the aseptic instructions in Section 10.3.1 above, and to then follow this procedure:

1. Aseptically place the carriers, two per vial, into three 1.5 ml Eppendorf vials.
2. Add 1 ml CVD nucleus counting kit (provided with your BelloStage® system).
3. Vortex the vials for approximately one minute.
4. Incubate the vials in the CO_2 incubator at 37℃ for two hours, vortexing the vials every 30 minutes during the incubation period.
5. Count the nuclei in the solution using a hemocytometer (see *Figure 18*).
6. Average the cell count per carrier, then multiply that average value times 860 (the average number of BioNOC® II disks in a BelloCell® bottle) to calculate the probable cell population.

> ***NOTE***
>
> **This is not an accurate method to assess cell viability.**.

Figure 18. Nuclei of Vero Cells (Stained with crystal violet dye)



Nuclei show bright purple color and oval shape in phase contract microscope

10.3.3.2 Counting Cells by Trypsinization

Another method to count cells is to randomly pick six BioNOC® II disks from the carrier disk chamber, following the aseptic instructions in Section 10.3.1 above, and to then follow this procedure:

1. Aseptically place the carriers, two per vial, into three 1.5 ml Eppendorf vials.
2. Add 1 ml PBS/EDTA (0.02%) to the vial, and wash the carriers by inversion 2~3 times.
3. Remove the PBS/EDTA. Repeat the wash at least twice.
4. Add 1 ml trypsin (0.05% trypsin/0.02% EDTA) to the vial.
5. Incubate at 37℃ for 10 mins.
6. Flick the vial 10 more times by fingers until the solution becomes turbid.
7. Count the cells and viability with trypan blue dye exclusive method in the solution using a hemocytometer.
8. Average the cell count per carrier, then multiply that average value times 860 (the average number of BioNOC® II disks in a BelloCell® bottle) to calculate the probable cell population.

NOTE

Cell count might not be accurate if cells cannot release from the carrier efficiently.

10.3.4 Harvesting Cells

BIOHAZARD WARNING!
The user is responsible for following local guidelines for handling hazardous waste and biohazardous materials that may be generated from the use of this equipment.

When the culture run is terminated, users may want to harvest cells or cell components for use elsewhere. One solution is to disassemble BelloCell®-500 bottle by **BOTTLE OPENER*** to BioNOC® II carriers, and then harvest the cells or cell components either by adding lysis buffer or freeze-thaw procedure. ***Bottle Opener** can be purchased from CESCO Bioengineering.

NOTE
The above harvesting procedure is not aseptic

For aseptic cell harvest cells or cell components aseptically, we recommend the following procedure:

1. Discard the culture media from the BelloCell®-500 bottle. Observe proper handling and disposal procedures if the media contains potentially biohazardous material.

2. Add 500 ml of pre-warmed PBS/EDTA to the BelloCell® bottle. Install the bottle in the BelloStage unit. Program the Up/Down Rate at 2.0 mm/s and start the START key. Stop the compression after 5 mins; remove the bottle and asceptically open it to discard the washing solution.

3. Repeat step 2 twice more at least..

4. Add 400 ml* of pre-warmed trypsin/EDTA solution (0.05%/0.02%) to the bottle; Incubate for 10~15 mins. *CESCO provides **CELL DISSOCIATION SUPPORTER** to reduce trypsin requirement to 250 ml.

5. Aseptically discard the enzymatic solution, and incubate for 10~15 mins more.

6. Tap the bottle sharply against your palm or a soft, round counter edge, at the matrix chamber level, for 3 ~5 minutes, to force cells to release from the carriers and the matrices they have created.

7. Add 500 ml Culture medium or PBS containing FBS or trypsin inhibitor to the bottle.

8. Install the bottle on BelloStage® in a CO_2 incubator. Run the unit for 2~3 mins at the Up/Down rate at 2.0 mm/s.

9. Remove the bottle and aseptically harvest the cell-laden solution, and then harvest the cells by centrifuging the solution.

10. Repeat step 6-9 at least three times until the cell residue harvested falls below the desired concentration.

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.

11 MAINTENANCE

Periodic maintenance is essential to the proper operation of your BelloStage® system.

11.1 Cleaning

Periodically clean the BelloStage® unit and controller by wiping the outside surfaces with a soft cloth moistened with a 70% alcohol solution.

CAUTION!

The exterior surfaces of the BelloStage unit and controller are coated with an anti-rust material. Never use any abrasive materials and be careful not to scratch the exterior finish.

11.2 Maintenance

We recommend that you grease the BelloStage® unit's platform **ball screw** (see *Figure 19a*) once every three months, to keep it operating smoothly:

1. Turn off and unplug the BelloStage® unit.
2. Remove the unit from your incubator.
3. Slowly and carefully apply approximately 0.5 ml of grease, using a user-supplied syringe, to the platform **ball screw**, from the bottom up (*see Figure 19a*).
4. Make note on the maintenance card (see *Figure 19b*)

Figure 19a. Greasing the Ball Screw

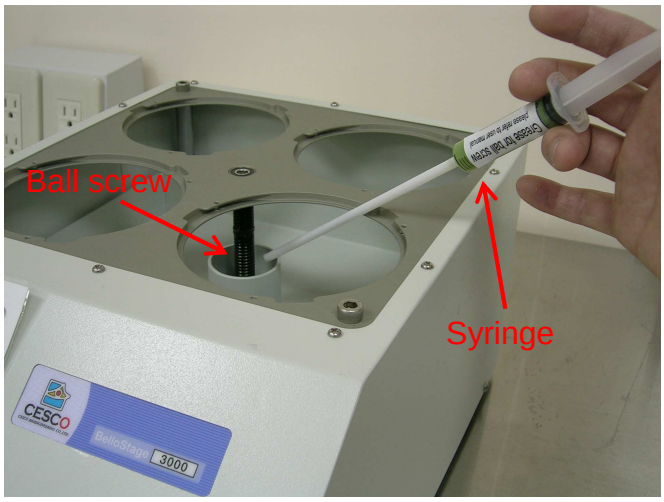
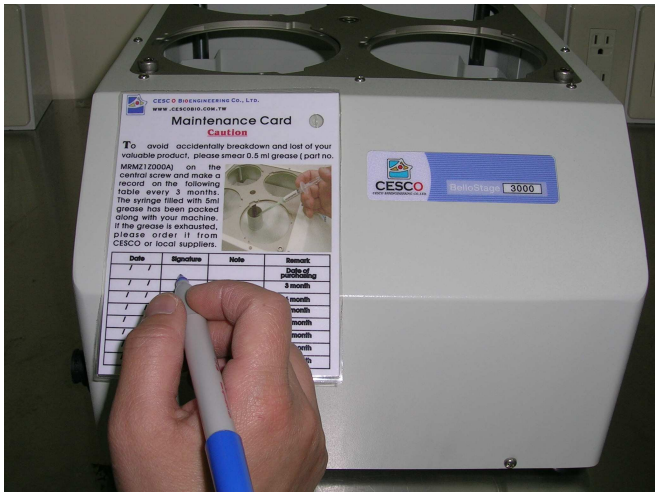


Figure 19b. Recording after Maintenance



11 TROUBLE SHOOTING

CAUTION!

Cells could keep activity even have been shut down for over night. Please try to turn on the machine or change the bottles to another equipment immediately.

Index	Possible Cause	Solutions
Continuous alarm, 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. Loosen on signal cable between control box and the BelloStage unit.	1. Remove the rust and grease the ball screw. Be sure to grease the ball screw every 3 months. Remove the BelloStage unit from the incubator if not use for a while. 2. Replace the step motor. 3. Make sure the 9-pin signal cable between control box and platform unit is well connected.
"UPPER LIMIT ERROR" Platform can not reach the upper limit	1. Failure on the upper limit optical sensor	1. Clean the upper limit optical sensor. Make sure no dirt on the sensor surface
Controller looks normal, but unit cease movement	1. Burn down of the 6V DC-DC circuit 2. Malfunction of the step motor	1. Replace the 6V DC-CD circuit 2. Replace the step motor
Random codes on 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 single power source of the equipment
Control box can not hold on the incubator	1. Dirt between the magnetic backplate 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
If you are unable to solve the above problems, please contact local CESCO distributor or e-mail: info@cescobio.com.tw		

13 Question & Answer

1.Q: Can I harvest cells after culture?

A: Yes, you can harvest cells after culture terminated. **BelloCell®-500** can be disassembled and the carrier box can be taken out for this purpose. You can also harvest cells aseptically without disassemble the bottle. Please refer to the instruction manual for detail instruction.

2.Q: How can I observe cell growth on the carriers?

A: The carriers are made of porous nonwoven fabrics. The common staining solution is hematoxylin, coomassie brilliant blue G, etc. Please follow the instruction manual for each staining application. Then you can see the cell morphology in carriers under a microscope.

3.Q: Can I take out carriers for sampling during culture?

A: Yes. **BelloCell®-500** model is equipped with a sampling window on the carrier box. With long-arm forceps, users can take out carriers through aseptic techniques during culture.

4. Q: Can I count the cell population during culture?

A: Yes. After take out carriers through aseptic techniques during culture, users can measure the cell population through crystal violet dye nuclear count method. Each BelloCell bottle contains 865±5% carriers. User can estimate the total cell population by timing back the total carrier number. Cesco also provide CVD nucleus count kit.

5.Q:Can I culture cells by using serum-free medium in BelloCell?

A: Most serum-free media can be applied to **BelloCell** culture. If the medium allows cells to attach or partially attach (50% suspend, 50% attach) on typical T-flasks, then it should be able to be used in the **BelloCell®-500** system. However, for loosely attached cells, we recommend to use **BelloCell®-500P** instead of **BelloCell®-500**.

6.Q: What is the BioNOC II carriers in BelloCell ?

A: BioNOC® II carriers are made by FDA approved 100% PET fabrics. They were processed through a special surface treatment to make it biocompatible. The carriers provide around 2,400 cm^2/g specific surface area for cell growth. One **BelloCell®** contains 5.5 g of the **BioNOC® II** carriers.

7.Q: Can I obtain BioNOC® II carriers solely for other purposes?

A: Yes, you can purchase **BioNOC® II** carriers. They can be applied to our **TideCell®** bioreactor or other packed-bed type bioreactors.

8.Q: Can I reuse BelloCell® ?

A: BelloCell® is designed for one time use only, and it cannot be sterilized by autoclaving. Reuse **BelloCell®** is not recommended. A **BelloCell®** can sustain cell growth for months without causing distortion or contamination with an appropriate and aseptic operation.

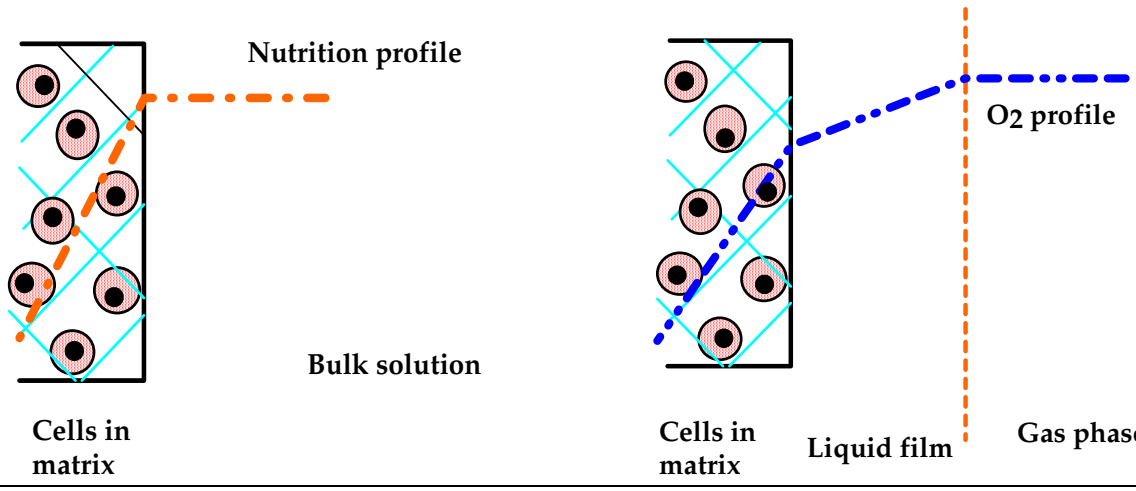
9.Q: Can I use BelloCell for long-term culture?

A: BelloCell has passed the test of 200,000 times compression, which is equal to 3 months at the highest speed or 33 month at the lowest speed. Since the cells are immobilized in the carrier box, user can culture the cells as long as providing them enough nutrients.

14 Reference Guide

The patented BelloCell® contains two chambers: the upper chamber contains a porous fibrous matrix mounted inside where the cells are embedded; the lower chamber contains a compressible bellows and has culture medium inside. The BelloStage® compresses the lower bellows in order to expel the culture medium out of the lower chamber and raise the culture medium level to submerge the matrix in the upper chamber. Alternatively, the BelloStage®-3000 lowers compressible bellows in order to lower the culture medium level to expose the matrix to the air. The matrix containing carriers packed in the culture vessel is thus exposed and submerged in the culture medium alternatively controlled by the moving rate of the platform of BelloStage® (see *Figure 21*). During the exposing phase (see *Figure 20*), the cells embedded in the porous carriers will not expose directly to the gaseous environment but through a thin liquid film as shown in the following schematic diagram. Not only this would not cause the damage of the culture cells, but also facilitate the most efficient oxygen transfer from air to the cells. During the submerging phase (see *Figure 20*), the cells expose to the new liquid surface and facilitate the uptake of nutrients from the medium (such as glucose, glutamine and growth factors, *etc*.) and removal of the wastes (such as ammonium, lactic acid, *etc*.) from the cells.

Figure 20. Cross-Section view of liquid (left) and gas (right) transfer across the carriers and cells



Oxygen transfer limitation is always the threshold of achieving high cell density in cell culture. In the stirring tank bioreactor system with or without use microcarriers, it requires high air sparging and agitation rate to achieve high oxygen transfer but it also generates high shear stress and foaming problem, which are detrimental to animal cells. The hollow fiber bioreactor system is designed to generate lower shear stress and perform perfusion culture, but it requires exterior oxygenation system and high circulation rate to provide sufficient oxygen supply. Furthermore, the axial non-uniform distribution of cells and proteins along extracapillary (EC) space because of convective flow in EC may result in serious sedimentation and poor oxygen transfer. Some commercial packed bed bioreactor system is designed on basis of airlifting principle combined with modified impeller to promote gentle liquid circulation through annular packed bed with low shear stress but good oxygen transfer. However, the height of packed bed limits the industrial scale-up capability for this type of bioreactors. In order to minimize the disadvantages and retain the advantages of the commercialized bioreactors aforementioned, CESCO Bioengineering Co. has developed **BelloCell®** and **TideCell®** bioreactors. In BelloCell® and TideCell® a massive oxygenating surface area was created with simple medium movement relative to the cell embedded in matrix for highly efficient oxygen transfer without any agitation and air sparging. BelloCell® is a disposable bioreactor mainly designed for simple, efficient and economical laboratory use while TideCell® was for pilot and production plant application.

Figure 21. Diagram showing the nutrition and aeration phase during culture in BelloCell system