

Principle

BelloCell[®] is a disposable bioreactor capable of high density cell culture for protein expression, virus and monoclonal antibody production.

BelloCell[®] is designed based on the concept of bellows-induced alternation flow of media and air through porous matrices, where cells reside and grow, providing a low shear, high aeration and foam free culture environment.

BelloCell[®] bioreactor consists of two components: a sterile, single-use culture bottle BelloCell[®] and a bellows compressor BelloStage[®].

During usage, BelloCell[®] bottle is partially filled with media and inoculated with cells.

The media are raised and lowered alternatively to submerge and expose the matrices, creating a dynamic interface between air and media on cell surface to maximize nutrient uptake and oxygen transfer.

Because of its high efficiency in nutrient and oxygen transfer, one BelloCell[®] bottle, with specific surface area of 15,600 cm², is capable of producing cell mass comparable to 20 to 30 roller bottles.

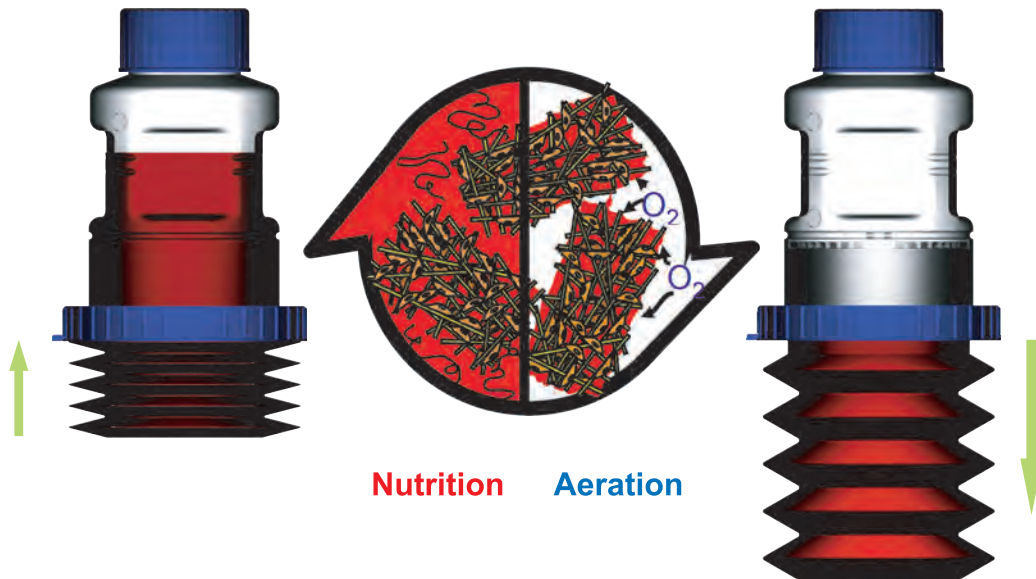
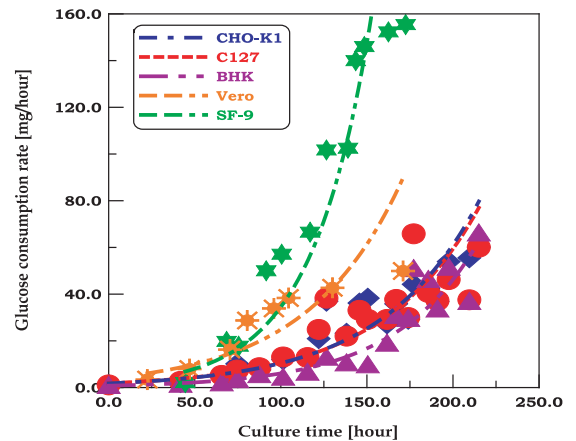
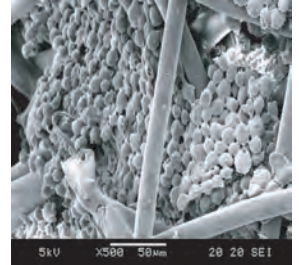
Since the matrix surface is specially treated, BelloCell[®] can grow most anchorage-dependent cells and allow easy harvest of whole cells, cell components or secreted proteins.

Features & Advantages

- Pre-sterile, ready-to-use, disposable
- Low shear stress, foam-free, no O₂ limitation
- Large surface area for high density cell culture
- Compatible with most serum free media
- Be able to collect whole cells or cell components

Applications

- Mammalian and insect cell culture
- Protein and virus production
- Monoclonal antibody production
- Proteome research
- Drug discovery
- Pharmacokinetics study
- Gene and cell therapy



Cells remain entrapped in the disk bed, as the BelloCell[®] bottle is compressed, media is forced up through the disk bed, supplying the cells with nutrients and facilitating removal of metabolic waste

When the BelloStage[®] platform lowers, the bottle fully expands and media recedes, exposing carriers to air to facilitate aeration.

Cells are protected by the disk without interference by the medium flow. Low shear stress, no oxygen limitation, no foaming problems.



Each BelloStage® console accommodates up to four disposable bottles making this an ideal screening device to test varying medium formulations or cell lines.

No steam or water lines, autoclave or utilities required, just a power outlet and CO₂ incubator

Simple to operate – virtually no learning curve

Controller- adjusts nutrient and gas exchange of the bottle

Magnetized controller enables convenient positioning by attaching on the outside surface of the incubator.

Useful for batch and semi-batch operation where process components are easily traceable.

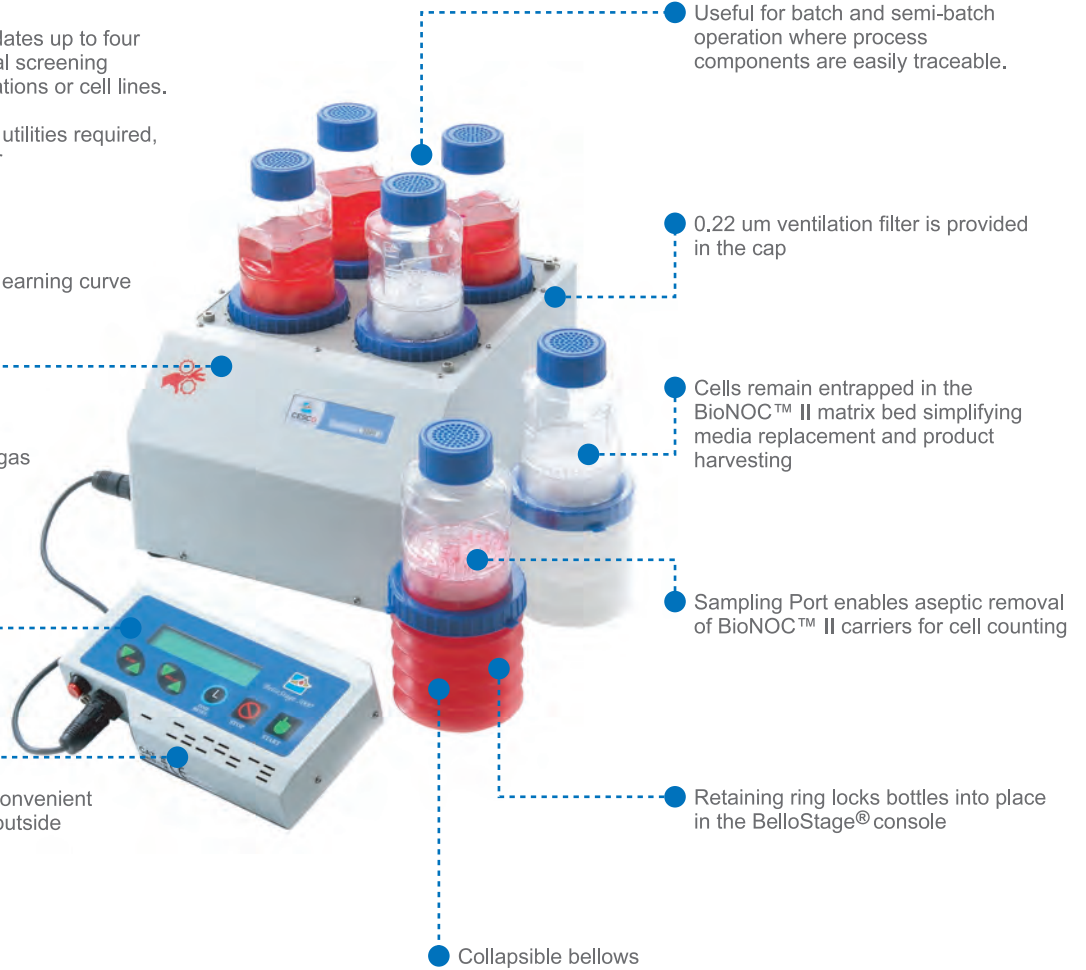
0.22 um ventilation filter is provided in the cap

Cells remain entrapped in the BioNOC™ II matrix bed simplifying media replacement and product harvesting

Sampling Port enables aseptic removal of BioNOC™ II carriers for cell counting

Retaining ring locks bottles into place in the BelloStage® console

Collapsible bellows



Extremely compact system fits in most standard CO₂ incubators

Bright and large display is easy to read.

UP: 1.5 ml/s
T_H: 04 M 04 S

Useful for continuous operation where process components are easily traceable.

Tubing set with peristaltic pump head enable recirculation and continuous feeding of nutrient

BelloFeeder enables pumps operation with individual programming setting

Autoclavable pump head supports consistent feeding rate with calibration free



Features



Easy-to-Use, Disposable, Space and Labor-Saving Device

Disposable, ready-to-use, virtually no start-up time, and no learning curve

Highly scaled down product. One bottle provides 15,600 cm² specific surface area, enabling >1x10¹⁰ cells with four bottles in a 8.6" x 11.7" footprint and a 6 cu. ft. incubator.

Produce milligrams to grams of proteins, monoclonal antibodies, 10¹¹~10¹² pfu viruses, whole cell or cell components, eliminating the need to maintain numerous spinners, dozens of roller bottles, and hundreds of T-flasks.

Scale up by directly multiplying number of bottles, eliminating the variation, uncertainty and time spent during scale-up process development. Yield from a dozen of bottles could replace 200 roller bottles that could be sufficient for most clinical trial studies.

No Learning Curve, Cell Culture Made Simple

Operation and medium exchange protocol is similar to tissue culture flasks and roller bottles but can replace dozens of roller bottles or hundreds of tissue culture flasks in just one 1 L vessel.

Specially designed for laboratory researchers to fit into original T-flask protocol without requiring any engineering background, and allows researchers to focus more of your valuable resources on fundamental research.

Cells become entrapped in the matrix bed, where they remain throughout the process, simplifying media replacement and product harvesting.

High Yield, Special B_H Function to Boost Protein Expression

Special B_H function (increasing bottom holding time) restricts cells from over-growing and enhance protein expression over 3 folds. Simple, and efficient. It also enables higher protein concentration and save culture medium during culture.

Various Bottles Cover Broad Range of Application

Various bottles constructed either with different culture matrix or different purpose of design ensure a broad range of application. Example of the bottles includes special dissolvable matrix BioNOC™ D in BelloCell®-500DP bottles, which can dissolve the matrix after cell culture, enable to harvest high quantity of cells, viruses, and non-secret protein without requiring enzymatic treatment that might injure the valuable products.

The modification of BelloCell®-500AP enables to harvest matrices, enhances cell seeding, and virus infection efficiency.

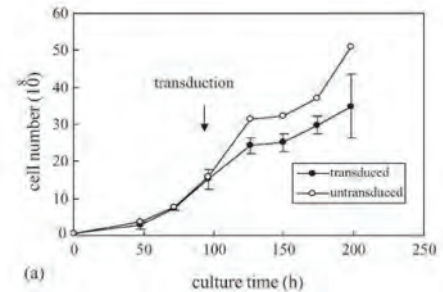
Many features are included in the broad range of BelloCell® bottles.



Protein Expression by Recombinant Viruses

BelloCell[®] system provides a simple tool to produce hundreds of ug to multi-mg of recombinant protein through recombinant viruses such as vaccinia, baculoviruses or other recombinant viruses. The single use, and disposable design of the BelloCell[®] bottle provides protection to lab workers to deal with those recombinant viruses while the simple, compact, and space saving nature of the bottle reduces the possible risk during operation.

- Produce hundreds micrograms to multi-milligrams of recombinant protein through the transfection or transduction of recombinant viruses.
- Produce $\sim 4 \times 10^9$ transfected cells in one single bottle, equivalent to 10~20 roller bottles. Expressed protein is more concentrated than conventional culture systems. Reduce labor and space for producing protein of interested in large quantities.
- Ready-to-use and disposable design of the BelloCell[®] bottle protects lab workers who handle biohazard recombinant virus materials.

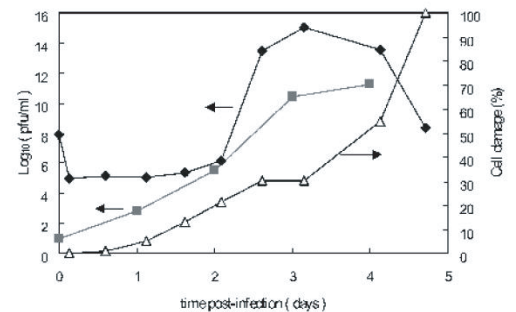


Transfect recombinant baculovirus to BHK-21 cells with $\sim 90\%$ transduction efficiency. Start infection at 1.5×10^9 cells per bottle, cells continue to express protein and propagation and reach final transduced cell density to $\sim 4 \times 10^9$ cells. [3]

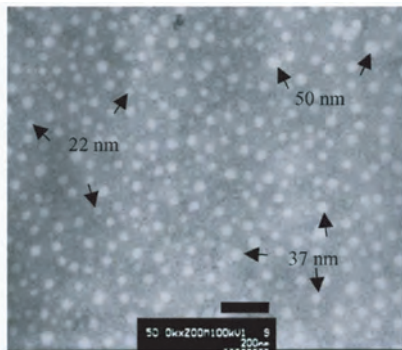
Virus Production

Due to the high cell density in the BelloCell[®] bottle, users could produce viruses at a concentration one log magnitude higher than conventional culture systems. This will be beneficial to prepare virus stock, or to manufacture combinatory vaccines without over diluting the virus titer. However, due to the over 10 fold increase of the cell density in the bottle, control of pH within proper range during post-infection period is critical to obtain satisfactory result. Examples obtained in BelloCell[®] system for virus production is log 12~13 pfu for baculovirus, log 13~14 vp for adenovirus, log 11~12 pfu for Japanese encephalitis virus, and log 11 pfu for WEE virus.

- Produce log 11 to 13 pfu viruses from one single bottle
- 1 log increase in virus concentration, suitable for preparing combinatory vaccines or virus stock
- Ready-to-use, disposable and one bottle operation instead of dozens of roller bottles. Reduces the risk during handling biohazardous virus materials.



Time course profiles of the virus titers produced in BelloCell[®] (\blacklozenge) as well as in spinner flasks (\blacksquare), and percentage of cell damage in the BelloCell[®] system (\triangle) assessed by LDH activity variation.



Virus particles produced from BelloCell[®] bottles [4]

References

1. Ing-Kae Wang et al, Journal of Biotechnology, 418-428, 121, 10 Feb, 2006
2. Lewis Ho et al, Cytotechnology 45: 117-123, 2004
3. Yi-Heng Chen et al, Journal of Biotechnology, 135-147, 118, 2005
4. Yu-Chen Hu et al, Cytotechnology 42, 145-154, 2003

for more literatures, application notes and protocols for the above application examples, please visit www.cescobio.com.tw

Literature Support

1. "Japanese encephalitis virus production in Vero cells with serum-free medium using a novel oscillating bioreactor", Hiroko Toriniwa, Tomoyoshi Komiya, Department of Research and Development, Research Center for Biologicals, The Kitasato Institute, Biologicals in press, 2007
2. "A novel control scheme for inducing angiostatin-human IgG fusion protein production using recombinant CHO cells in a oscillating bioreactor", Ing-Kae Wang, et al., Journal of Biotechnology. Available on line 12 September 2005
3. "Production of recombinant human growth hormone", Fabienne Anton, et al. Institute fur Technische Chemie, Callinstr, Hannover. ESACT 2005
4. "Baculovirus-mediated production of HDV-like particles in BHK cells using a novel oscillating bioreactor", Yi-Heng Chen, et al., Journal of Biotechnology. Available on line 13 June 2005
5. "Cultivation of HEK 293 cell line and production of a member of the superfamily of G-protein coupled receptors for drug discovery application using a highly efficient novel bioreactor", Lewis Ho, Cynthia L. Greene, Anne W. Schmidt and Liang H. Huang, Cytotechnology 45: 117-123, 2004
6. "Effect of 3-D culturing of HEK-293 on a fibrous matrix and production of G-protein coupled receptors for Drug Discovery", C.L.Greene*, L. Ho, A.W. Schmidt, L.H. Huang, Poster in 2004 Annual Meeting of the Society for Industrial Microbiology (SIM), July. 25-29, 2004.
7. "High-density cultivation of insect cells and production of recombinant baculovirus using a novel oscillating bioreactor" Yu-Chen Hu*, Jen-Te Lu and Yao-Chi Chung Department of Chemical Engineering, National Tsing Hua University, Hsinchu, Taiwan 300, ROC; Cytotechnology 42: 145-153, 2003.
8. "Novel Animal-Cell-Culture Device for Mass Production of Preteins and Viruses" King-Ming Chang and Lewis Ho Genetic Engineering News, Vol. 23, No. 15, Sep. 1, 2003
9. "Novel Disposable Bioreactor for Mammalian Cell Cultures and Virus Production", Yu-Chi Wang, King-Ming Chang, Fen-Lang Lin, Mei-Lin Wu, Lewis Ho*, Poster in BioPHEX, Oct. 22-24, 2002, Santa Clara Convention Center, Santa Clara, California
10. "Novel oscillating bioreactor BelloCell: Implications for insect cell culture and recombinant protein production", Lu J-T, Chun Y-C, Chan Z-R, Hu Y-C, Biotechnology Letters, 27:1059-1065, 2005

Disposable BelloCell® Bottles

– A complete product line of BelloCell® meets your special need



Different BelloCell® bottles cover 90% of applications in cell culture.

- Semi-batch or continuous culture
- Disposable or Re-useable
- Cesco' carriers or your own carriers
- Harvest cells with or without trypsin

Table 1. Selection guide

Application/Bottles	Secreted Protein, viruses (adherent cells)	Cell Harvest (for non-secreted protein, virus, or cells)	Carrier Harvest (for protein extraction, or reuse carriers)	Suspend cells
BelloCell® -500	●	●	●	●
BelloCell® -500P	●	●	●	●
BelloCell® -500A	●	●	●	●
BelloCell® -500AP	●	●	●	●

● : Best application

● : Applicable, but not optimal

● : Not applicable

Order information

Cell Culture Bottles	Cat. No.	Package
BelloCell® -500	BCB00500	4 bts/case
BelloCell® -500P	BCB01000	4 bts/case
BelloCell® -500A	BCA00500	4 bts/case
BelloCell® -500AP	BCA01000	4 bts/case



BelloCell[®] High Density Cell Culture System Complete

The BelloCell[®] Cell Culture System Complete contains all the necessary elements for users to start cell culture right after opening the package.

It is designed to support four BelloCell[®]-500 bottles with a 8.6' x 11.7' foot-print and fit inside a standard CO₂ incubator.

It contains a BelloStage[®] console with a magnetized controller, which can be conveniently stored and accessed from the outside wall of the incubator.

Features

- Includes a BelloStage[®] compressor, and necessary accessories for you to start up culture right after receiving the package.
- Extremely compact system fits in most standard CO₂ incubator.
- Controller with large and bright display and large touch panel. Easy to read and operate.
- Suitable for batch or semi-batch operation.
- Suitable for most protein and monoclonal antibodies production applications.



Order Information

Product Name	Catalogue No.	Package
BelloCell [®] System Complete	B S 4000	1x BelloStage [®] -3000 Machine 1x GlucCell [®] Glucose Meter 50x Glucose Test Strips 1x Manual CD



BelloCell[®] Continuous Cell Culture System Complete

The BelloCell[®] Continuous Cell Culture System Complete provides the similar features as BelloCell[®] Cell Culture System Complete but with additional a BelloFeeder[®] pump and tubing sets to provide continuous medium exchange in the BelloCell[®]-500P bottles.

Each BelloCell[®]-500P bottle equips additional inlet and outlet line for medium recirculation.

Each bottle can link to an extra medium vessel and provide continuous exchange of nutrient, eliminating the labor for medium exchange and avoiding possible shock to cells during culture.

Features

- Each package contains a BelloStage[®] -3000 compressor, a BelloFeeder[®] pump module, two tubing complete sets and accessories for users to start up culture right after receiving the package.
- Each bottle connects with independent medium reservoir to eliminate cross-contamination.
- Programmable and ON/OFF pumping control simplifies the setting of recirculation rate for each bottle.
- One BelloFeeder[®] pump module enables to operate up to 4 pump heads with individual micro-processor control.
- Suitable for cell mass production, cell component production, virus production, or protein expression.



Order Information

Product Name	Catalogue No.	Package
BelloCell [®] Continuous System Complete	7	1x BelloStage [®] -3000 Machine 1x BelloFeeder [®] -1000 Pump 2x Tubing Complete Set 1x GlucCell [®] Glucose Meter 50x Glucose Test Strips 1x Manual CD



Accessories

- Complete accessories support your working with BelloCell® system

Tubing Complete Set

Tubing Complete Set includes pre-assembled tubes, reusable pump head and head plate with a sampling port to support the continuous culture in BelloCell®-500P system. It renders autoclave at 121° C for 30 minutes.



Crystal Violet Dye Nucleus Count Kit

Crystal Violet Dye Nuclei Count Kit contains crystal violet dye, citric acid and detergent to disrupt cells and release cell nuclei for cell count.

This provides an efficient reagent for cell count in porous matrix.



Order Information

Product Name	Catalogue No.	Package
BelloStage® -3000 Machine	3	1x main console 1x control box 1x 100~240V power adapter 1x signal cable 1x Manual CD
BelloFeeder®-1 00 Pump	1 00	1x BelloFeeder®-1 00 pump
Tubing Complete Set	1	1x Disposable Tubing Accessory 1x pump head 1x head plate
Crystal Violet Dye Nucleus Count Kit		Volume: 100 ml/bt.

