• How to start a culture •

1. First of all, please read through the Instruction Manual before start your culture.
2. For the culture that already has Application Notes found in the CD or in our web site (www.cescobio.com.tw), it's a good beginning for your reference.
3. For the first time doing culture in BelloCell, please follow the tips bellow:

(A). **Higher cell density will shorten the lag phase during culture.** For a smooth run in a BelloCell-500, one may require at least 5×10^7 to 2×10^8 inoculums for mammalian cell culture or 1.5×10^8 to 2×10^8 inoculums for insect cell culture. It may require three T-150 flasks or one roller bottle or one 250 ml spinner flask to prepare enough cells as inoculums.

Cell Type	Inoculation density (cells/bottle)
CHO, VERO, BHK, C-127, RK-13, HEK-293A	1×10^8
HEK-293, Sf-9, Hi-5, Sf-21, Hybridoma	$1.5\sim2.0\times10^8$

Do you have any problems on preparing those cells?

(B). **Whether your culture can be adapted into BelloCell depends on the culture medium.** Choose the right media that can be used in BelloCell-500 system. Most suspend cells can be immobilized in our matrices even in serum-free culture. You can directly check the applicability by starting culture on BelloCell-500. However, the most economical way to identify if your cells can be attached on the matrices is to test whether your cells can be attached or partially attached (~50% attach with other ~50% suspend) on T-flasks. T-flasks from Nunc or Corning are better for the choice. Those culture media that cannot support cell adherence will have difficulty to be applied in the BelloCell system. Serum-free media that may be suitable for applying in our system are listed below. Dark blue characters are the one that have been tested and worked well in our system.

Cell Line	Serum-Free media
CHO	**CHO-S-SFM II (Gibco)**, CHO III A (Gibco), CHO-A-SFM (Gibco) , **ExCELL 301 (JRH)**, **HyQ PF-CHO (Hyclone)**, **HyQ-CCM 5 (Hyclone)**, **HyQ SFX-CHO (Hyclone)**, IS-CHO-CD (IRVINE), **proCHO4cdm**
HEK-293	EX-CELL 293 (JRH), Pro293a-CDM (CAMBREX), **HyQ SFM4 HEK293 (Hyclone)**
VERO	**VP-SFM (Gibco), Plus VERO (CESCO),** ICN-VERO (ICN), PEEK-1 (Biochrom),

Cell Line	Serum-Free media
Hi-5	**EX-CELL 405 (JRH)** **Express Five (Gibco)**
Hybridoma/NS0	EX-CELL 610-HSF (JRH)

Do the cells attached or partially attached on T-flasks in your culture?

(C). Inoculation set up is recommended as below:

UP/DOWN Speed (mm/s)	Top/Bottom holding time (T_H/B_H) (sec)
2.0/2.0	20/0

Usually, within 2 to 4 hours, above 90% cells can be immobilized in the matrices. During inoculation, please distribute seeds directly on the top of the matrix basket, bring the bottle to the BelloStage console and start operation immediately. Avoid shake or swirl the bottle after seeding the cells. Immobilization efficiency depends on the correct protocol during seeding and will affect the cell growth rate significantly. Well begun is half down!

(D). PH in culture medium higher than 7.6 may slow down the attachment of cells in BelloCell system. If the culture medium contains 2.2 g/L $NaHCO_3$, please set CO_2 at 5%. Culture medium containing 25 mM HEPES can stabilize the pH during culture. Please adjust the culture medium at the range of between 7.2 to 7.4 for mammalian cell culture; 6.2 to 6.4 for Sf-9 insect cell and 6.4~6.5 for Hi-5 cell culture.

What's the initial pH, $NaHCO_3$ and HEPES concentration in the culture medium? What's the % of CO_2 in the incubator?

(E). Medium consumption rate depends on your process. For virus production in semi-batch culture, one may require 1 to 3 L medium. For continuous collecting conditioned culture medium for secreting protein production, one may require medium for 5 to 10 L to extend the culture above 15 days. Cells in BelloCell can be retained for months and sufficient supply of nutrient is one of the key factors.

Have you prepare enough medium for the run?

(F). To set up culture parameter, we suggest you to try 1.0 mm/s up/down rate and 0/1 mins up/down hold time at the beginning; ***Increase speed facilitates the mixing of culture medium but also possibly increase the shear stress to cells; increase bottom holding time facilitates the aeration efficiency but also reduce the mixing frequency of culture medium.***

Below are the recommend parameters for certain cell lines.

Growth-associated application (production is proportional to cell growth. For example, those who want to collect cell mass, cell components, non-secreted protein, or the protein production depends on growth activity of cells)

Up/Down Speed (mm/s)	T_H/B_H	Cells/cell line
1.5/1.5	10 s/1 min	HEK293, BHK, C-127, VERO, CHO, Sf-9, Hi-5

Non-growth-associated application (production is higher when cells are slow growing or cease growing)

Up/down Speed (mm/s)	T_H/B_H	Cells/cell line
1.5/1.5	10 s/30~60 mins	BHK, CHO

Loosely attached cell line

Up/down Speed (mm/s)	T_H/B_H (min)	Cells/cell line
2.0/2.0	2/30~60	Hybridoma

Avoid disturb the bottle during culture for loosely attached cell lines. Use perfusion system BelloCell-500P is highly recommended.

If you don't know how to choose the parameters, try the 1.0 mm/s up/down rate and 0/1 mins top/bottom holding time (T_H/B_H) at the beginning for all cases.

What's your proposed set up parameters?

(G). To efficiently determine the best time for medium replenishment is tricky in almost every culture process. In BelloCell-500 culture, whether the medium should be changed can be determined by the criteria below:

i、 glucose concentration below 0.5~1.0 g/L (initial glucose concentration should be 2.5 ~ 3.0 g/L)

ii、 pH below normal range, but glucose concentration still above 1.0 g/L → adjust CO_2 ; if CO2 concentration in incubation has been set to 0 → add buffer or change medium.

iii、 For continuous culture over weeks or months, replenish medium once a day or per two days (0.25~0.5 L/day) is necessary to keep cells stay at optimum condition. Increase B_H will decrease the nutrient consumption rate and be able to extend the medium exchange frequency. If the media is rich enough and does not require frequent media exchange, please monitor and adjust pH by 7.5% $NaHCO_3$, 1 M HEPES or 1 M Bis-Tris (insect cells).

Do you have problems to monitor those parameters?

(H). Because the high cell density in BelloCell culture, the pH in medium may drop sharply at the later stage of cell growth. **Sequentially adjust the CO_2 concentration in incubator is necessary for mammalian cell culture in BelloCell-500 system if initial glucose concentration is above 3.0 g/L. For BelloCell-500P, pH could be controlled by increasing medium circulation rate without requirement to adjust CO2.** Once the CO_2 concentration has been adjust to 0 and pH still below normal range, increase NaHCO3 concentration up to 3.7 g/L and/or add HEPES to stabilize the pH. For insect cell culture, add 1M Bis-Tris is a good strategy to stabilize pH.

Increase up/down speed and bottom holding time will help to expel CO_2, reduce lactate production and thus can further stabilize pH in culture medium.

How are you going to control the pH of your cell culture medium?

Do you have those buffers at hands?

(I). Estimating cell numbers during BelloCell culture has two ways in our system. One may pick matrices in the BelloCell by sterilized long-arm forceps and estimate the cell population by crystal violet dye (CVD) nuclei count method. Each BelloCell contains 865±1% disks. One

can count cells in the sampled disks and calculate back to total cell population. One may also estimate the cell growth by calculating the glucose uptake rate (GUR). Measure glucose concentration every day or 6~8 hours after medium replenishment. Under similar culture environment, GUR is proportional to cell population. The equation of GUR is (glucose at time 1 – glucose at time 2)÷(time 1-time 2)×culture medium.

Cesco also provide glucose meter and CVD nuclei count kit for users. Please find detail from Cesco's website: www.cescobio.com.tw

Do you have biochemical analyzer in your lab?

Which method do you choose to measure the cell population during culture?

(J) BelloCell allows users to harvest cells. The sub-optimal cell harvesting protocol can be found in the CD or from our web site: www.cescobio.com.tw

(K). Basic requirement for a BelloCell-500 culture:

BelloCell-500:

Items	Amount
BelloCell-500	1
BelloStage-3000	1
Incubator	1
Culture medium	500 ml at the beginning
Glucose meter or biochemical analysis system (GlucCell™ Glucose meter)	1
Crystal violet dye nuclei count kit	1
Long-arm forceps	1, sterilized
T-flasks, pipette, pipette-aids	As needed

BelloCell-500P:

Items	Amount
BelloCell-500P	1
BelloStage-3000	1
BelloFeeder pump or other peristaltic pumps	1
Tubing set	1
Reservoir vessel (2 L at least)	1
Incubator	1
Culture medium	2700 ml at the beginning
Glucose meter or biochemical analysis system (GlucCell™ Glucose meter)	1
Crystal violet dye nuclei count kit	1
Long-arm forceps	1, sterilized
T-flasks, pipette, pipette-aids	As needed

Please contact Cesco Bioengineering Technical support for any questions or comments.

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