HyClone™ media and supplements

Development of a fed-batch process using Cell Boost™ supplements

Cell Boost supplements are available as eight different concentrated feed formulations that can be applied for fed-batch cultivation. The methodology described here, enables a rapid, two-step procedure to set up a fed-batch protocol using the best performing supplements for a given cell line and basal medium. In the first step, the best performing Cell Boost supplements are selected in feed-spiked batch cultures. In the second step, the ratio of the selected Cell Boost supplements is fine-tuned in fed-batch cultivation to optimize cell growth and recombinant protein production.

Selection of the best performing basal cell culture medium

Prior to selecting feed supplements, it is advisable for a new cell line to screen several basal media for the best performing medium. In batch cultures, the medium that supports the highest cell growth and maximal product titer is selected for subsequent fed-batch development.

Step 1. Selection of the best performing feed supplements

Cell Boost supplements can be prepared in a range of differently concentrated stock solutions (Table 1). To streamline supplements that contain different (amounts of) nutrients, a molar ratio can be calculated according to the amino acid content. To select the best performing Cell Boost supplements for a given culture, the molar ratio helps to add equal amounts of different supplements in a study design based on design of experiments (DoE).

The best performing Cell Boost supplements are selected using a DoE study, and the experiments are prepared according to Figure 1. The supplements should initially be spiked to the basal medium, and cultures subsequently operated as, so-called, feed-spiked batch cultures. To determine the maximum amount of supplements that can be added to the basal medium, experiment number 16 is used as reference. The maximum amount of feed-spiking should be tested by the user through the addition of all Cell Boost supplements (with respect to their molar ratio) to the basal medium until the osmolality reaches an arbitrary threshold of 400 mOsmol/kg. For most cell lines, this limit indicates the maximal amount of nutrients that can be added to the basal medium. Above this limit, negative effects on cell growth and viability are expected. The maximum addition (Level +1) is kept constant for each supplement. The Level -1 indicates no supplement addition. The Level 0 indicates that 50% of the maximal amount of supplement is added to the basal medium (= 50% of Level +1).

Once all feed-spiked DoE experiments are prepared, the medium can be inoculated with the investigated cell line. For example, feed-spiked batch cultures can be performed in TubeSpin bioreactors (50 mL) at a working volume of 30 mL. At a seeding cell concentration of 3×10^5 c/mL, cells can be incubated in a shaker incubator at 37°C, 7% CO_2, and 90% relative humidity. Starting from Day 3, and then daily, cell concentration, viability, and antibody concentration are determined. In addition, metabolites (i.e., glucose, lactate, glutamine, glutamate, and ammonium) can be monitored. In this case, it is optional to keep the glucose concentration above a target concentration (e.g., above 3 g/L) by addition of a concentrated glucose solution (e.g., 250 g/L). The batch cultures are terminated once the viability drops below 60%.

The generated results of the feed-spiked batch cultures (i.e., peak cell concentrations, antibody titer, integral over the viable cell concentration, and cell-specific antibody production rates) are thereafter transferred to a statistic software (e.g., the MODDE™ software) to determine the best performing Cell Boost supplements by statistical means. In the next step, the optimal feed ratio is fine-tuned by applying the selected Cell Boost supplements.

Table 1. Molar ratio of amino acids in stock solutions of Cell Boost supplements

Cell Boost no.	Stock solution (%)	Molarity (mM)	Molar ratio
1	10	208.0	1.00
2	10	351.6	0.59
3	5	115.6	1.80
4	10	365.5	0.57
5	5	131.3	1.58
6	5	135.6	1.53
7a	18.1	603.0	0.34
7b	9.5	313.3	0.03

Note! For Cell Boost 7b, one tenth of the aount of Cell Boost 7a is used.

DoE design	Cell Boost 1	2	3	4	5	6	7a	7b
1	-1	-1	-1	-1	-1	-1	-1	-1
2	1	-1	-1	-1	-1	1	1	1
3	-1	1	-1	-1	1	-1	1	1
4	1	1	-1	-1	1	1	-1	-1
5	-1	-1	1	-1	1	1	1	-1
6	1	-1	1	-1	1	-1	-1	1
7	-1	1	1	-1	-1	1	-1	1
8	1	1	1	-1	-1	-1	1	-1
9	-1	-1	-1	1	1	1	-1	1
10	1	-1	-1	1	1	-1	1	-1
11	-1	1	-1	1	-1	1	1	-1
12	1	1	-1	1	-1	-1	-1	1
13	-1	-1	1	1	-1	-1	1	1
14	1	-1	1	1	-1	1	-1	-1
15	-1	1	1	1	1	-1	-1	-1
16	1	1	1	1	1	1	1	1
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0

Level -1 = no supplement addition. Level 0 = 50% of max. supplement addition. Level +1 = max. supplement addition.
Note! For Cell Boost 7b, one tenth of the amount of Cell Boost 7a is used.

Fig 1. Example of a DoE design to select the best performing Cell Boost supplements in feed-spiked batch cultures.

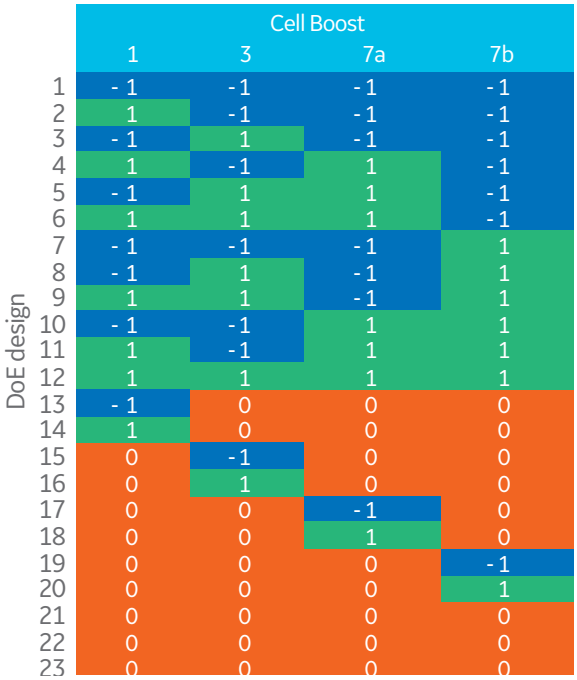
Step 2. Fine-tuning feed ratio of the best performing supplements

In this section, the feed ratio of selected Cell Boost supplements is fine-tuned. First, a master mix is generated by mixing the previously selected supplements according to their molar ratio. Second, a certain volume of this feed mix is added to the basal medium to simulate the conditions in a fed-batch culture after ten feed additions for ten consecutive days. This step will help to identify the maximum amount of Cell Boost supplements that can be added to a fed-batch culture as represented by the experiment no. 12 in the exemplified DoE design in Figure 2. Next, the osmolality of the sample is measured. The sample can be further diluted with basal medium to cover an osmolality range from at least 400 to 600 mOsmol/kg. These two values are important to know to define the upper (600 mOsmol/kg = Level +1) and lower (400 mOsmol/kg = Level -1) limits of feed mix that can be added to the fed-batch culture to avoid under- or overfeeding. The amount of feed for Level 0 is the mean of Levels +1 and -1 to reach a target osmolality of 500 mOsmol/kg after ten simulative feed additions. Finally, the amount of feed for Level +1, 0, and -1 needs to be divided by 10 to calculate the amount of daily feed addition.

Fed-batch cultures can be performed in TubeSpin bioreactors (50 mL) at a working volume of 25 mL. At a seeding cell concentration of 3×10^5 c/mL, cells can be incubated in a shaker incubator at 37°C, 7% CO_2, and 90% relative humidity. Starting from Day 3, and then daily, cell concentration, viability and antibody concentration are determined. In addition, metabolites (i.e., glucose, lactate, glutamine, glutamate, and ammonium) can be monitored. The glucose concentration should be kept above a target concentration (e.g., above 3 g/L) by addition of a concentrated glucose solution (e.g., 250 g/L).

Feed additions should be performed daily starting from Day 3. The sampling volume of each experiment should equal the amount of added feed to keep the volume constant and comparable in all experiments. The fed-batch cultures are terminated once the viability drops below 60%.

The generated results of the fed-batch cultures (i.e., peak cell concentrations, antibody titer, integral over the viable cell concentration, and cell-specific antibody production rates) are thereafter transferred to a statistic software (e.g. the MODDE software) to determine the best performing feed ratio of selected Cell Boost supplements by statistical means.



Level -1, 0, and +1 = amount of supplement to reach a target osmolality of 400, 500, and 600 mOsmol/kg, respectively, after ten feed additions.

Note! For Cell Boost 7b, one tenth of the amount of Cell Boost 7a is used.

Fig 2. Example of a DoE design to select the best performing Cell Boost supplements in feed-spiked batch cultures.

Optional protocol extension

Step 3. Fine-tuning of the feed strategy (constant versus dynamic)

In this section, the feed strategy of selected Cell Boost supplements is fine-tuned to compare several dynamic feed strategies to a constant/static approach. In total, four different feed strategies are investigated regarding their performance in relation to cell growth, antibody productivity, and product quality as follows:

1. ***Static strategy:*** a previously established amount of feed is added every day from Day 3
2. ***Concentration maintenance:*** key substrate* is kept at a certain concentration.
3. ***Predictive method:*** for future integrated viable cell count and specific consumption rates*.
4. ***Retrospective method:*** relative to the gain in viable cell concentration using the previously determined total average consumption rate of a key substrate*.

* As key substrate, glucose and glutamate are investigated.

All above described fed-batch strategies are performed in 250 mL shake flasks at a working volume of approximately 60 mL to ensure that sufficient material for sampling is available. Fed-batch cultures are operated as duplicate runs. The following applies for all fed-batch strategies:

- Feed start: on Day 3 and then every day thereafter.
- Sampling volume: to maintain a constant culture volume, the sampling and feed volume are equivalent.
- Cultures termination: once the viability drops below 60%.

Static strategy

This protocol will be a reference to the dynamic feed strategies. Feeding is performed by applying the best performing ratio of the selected Cell Boost supplements (determined during Step 2) at a constant feeding regimen. Additionally, a concentrated 250 g/L glucose solution is added to a concentration of 6 g/L if it drops below 3 g/L.

Concentration maintenance

Concentration maintenance is a method that is relatively simple to operate and will enable to conduct feed additions more dynamically. Two different key substrates (i.e., glucose and glutamate) are separately investigated and compared. A summary of this strategy is shown in Table 2.

Concentration maintenance of glucose:

Starting from Day 3, the best performing Cell Boost mix is added to the fed-batch cultures grown in basal medium to a target glucose concentration of 6 g/L. If the glucose concentration is above 6 g/L on Day 3 when the feed should be initiated, no feed is added to the culture. For this strategy, only the feed mix is used to maintain the glucose concentration and no concentrated glucose solution will be supplied.

Concentration maintenance of glutamate:

Starting from Day 3, the best performing Cell Boost mix is added to the fed-batch cultures grown in basal medium to a target glutamate concentration of 500 mg/L. If the glutamate concentration is above 500 mg/L on Day 3 when the feed should be initiated, no feed is added to the culture. For this strategy, a concentrated glucose solution (250 g/L) is used to maintain the glucose concentration of 6 g/L if it drops below 3 g/L.

Table 2. Concentration maintenance for fed-batch cultivation

	Glucose maintenance	Glutamate maintenance
Feed medium	Cell Boost supplements will be selected during DoE screening	
Feed start	Day 3, then daily	
Target concentration	6 g/L	500 mg/L
Glucose addition	None	Fill up to 6 g/L if lower than 3 g/L

Note! For Cell Boost 7b one tenth of the aount of Cell Boost 7a is used.

For glucose- and glutamate-based feedback additions, the amount of feed to be added to the culture to bring the target substrate to the desired concentration is calculated using Equation 1.

Eq. 1 mL to add

$$= \frac{[\text{target substrate (g/L)} - \text{measured substrate (g/L)}] * V_0 \text{ (mL)}}{\text{substrate concentration in feed (g/L)}}$$

Predictive method

The predictive method is a more laborious method by employing a dynamic fed-batch algorithm. Daily, the current cell concentration (X_0) and the previous cell concentration (X_{-1}) are determined to calculate the specific growth rate and predict the future cell concentration (X_{+1}) and a future integrated viable cell count (IVC). Subsequently, a nutrient consumption rate (R_1) will be calculated applying the current nutrient concentration (Y_0), previous nutrient concentration (Y_{-1}), previous feed volume (F_{-1}), feed concentration (Y_C), current working volume (V_0), and previous working volume (V_{-1}) to determine the total amount of feed per cell per unit time as shown in Equation 2.

Eq. 2 Nutrient consumption rate (pg/cell/day) = R_1

$$= (\text{previous} - \text{current} + \text{fed}) / (\text{integrated area})$$

$$= (Y_{-1} * V_{-1} - Y_0 * V_0 + F_{-1} * Y_C) / (IVC_0)$$

Applying the nutrient consumption rate, the final feed rate will be calculated according to Equation 3.

Eq. 3 Feed volume =

predicted consumption (mg) / concentration in feed (mg/L)

where

predicted consumption = consumption rate * predicted IVC –
(current nutrient concentration – desired nutrient concentration)

$$= (R_1 * IVC_{+1}) - (Y_0 * V_0 - (Y_S * V_0))$$

where

R_1 is the nutrient consumption rate as defined in Eq. 2.

IVC_{+1} is the predicted integral of viable cell concentration from the current to the next time point.

V_0 is the current culture volume.

Y_s is the desired setpoint of the nutrient concentration.

Note! The final term $(Y_0 * V_0 - (Y_S * V_0)$ represents a correction factor to adjust feed volume in case that the predicted nutrient consumption rate is inaccurate.

Retrospective method

The predictive method uses a dynamic protocol for feed addition that is based on previously determined average specific consumption rates of the used cell line, assuming the specific nutrient consumption remains relatively constant.

Based on previously determined substrate consumption, the substrate demand per cell and day will be converted to a distinct volume. Applying this, a certain feed volume will be added to the cultures relative to the gain in viable cell concentration (e.g., 1 mL of feed per 10^6 cells).

The calculation is based on a predetermined cellular consumption rate and concentration of either glucose or glutamate in the feed as described in Equation 4.

Eq. 4 mL to add

$$= [VCC] \times V_0 \times R / Freq \times [S]$$

where

VCC is the viable cell count (c/mL)

V_0 is the culture volume (mL)

R is the substrate consumption rate (pg/c/d)

Freq is the sampling frequency (1/day)

S is the substrate feed concentration (mg/L)

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