User Guide

Cell Culture Media 4CHO Sample Kit

Media-Screening for Fed-Batch Processes

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1. Introduction

This document provides instructions for the screening of our catalogue cell culture media for CHO cells in fed-batch processes. It gives guidance on the execution of the screening trials and the number of tests which may be performed from 1 L liquid medium depending on the scale of the selected test system.

The “Cell Culture Media 4CHO Kit” includes four different cell culture media samples in dry powder format for fed-batch processes: two basal media and two companion feeds (Table 1).

The two feeds are compatible with both basal media. The basal media can be combined with the two feeds individually or with a mixture of the two feeds at different ratios. It is recommended to test both basal media with individual feeds and feed blends (see 3.1).

Table 1: Cell culture media for fed-batch processes

Basal media	Cat. No.	Referring to in the following as
EX-CELL® Advanced CHO Fed-Batch medium	24366C	EX-CELL® Advanced CHO
Cellvento® 4CHO COMP*	103795	Cellvento® 4CHO
Feeds	**Cat. No.**	**Referring to in the following as**
Cellvento® 4Feed COMP*	103796	Cellvento® 4Feed
EX-CELL® Advanced Feed 1 (without glucose)	24368C	EX-CELL® Advanced Feed

* Comp. = compacted and means a proprietary manufacturing method generating homogenous media particles of a certain size which facilitate media hydration of dry powder.

2. Seed train and cell expansion

Before the actual media screening can be started, cells need to be generated in a sufficient quantity to be able to inoculate all the intended media evaluation tests at the required cell density.

Generally, cells are adapted to a new cell culture medium first before their growth and productivity are evaluated. **Direct media adaptation** can be a quick and suitable approach to amplify and adapt cells from a cell bank vial or actively growing culture to the cell culture medium of interest prior the actual media screening. Cells are directly seeded and expanded in the basal medium to be evaluated. For this application a certain amount of basal medium needs to be allocated for the cell expansion step. A protocol for direct media adaptation and cell expansion is provided in section 2.1.

Sequential media adaptation is also an effective but more elaborate and time-consuming method for cell adaptation. It relies on progressive sub-culturing in cell culture media mixtures of current and new medium with increasing ratios of the new cell culture medium. The cell culture is initiated and passaged under standard lab conditions using the current growth medium until the desired growth conditions (growth rate, cell density, viability) have been reached. Then, the cells are adapted stepwise to the new medium. If sequential media adaptation is preferred for certain reasons refer to Table 2 for reference. The adaptation guidance provided in Table 2 relies on regular sub-culturing of cells to maintain cultures in a logarithmic growth phase. This typically means that cells should be passaged every 2 to 3 days. At least two passages at each adaptation step are recommended to ensure that cells are appropriately adjust to their new cell culture media environment.

Table 2: Sequential media adaptation

Ratio of current medium to new medium (%)	Seeding density (cells/mL)	Acceptance criteria for next step
75:25	0.5×10^6	Normal cell doubling time; Viability >80%; at least two passages
50:50	0.5×10^6	Normal cell doubling time; Viability >80%; at least two passages
25:75	0.5×10^6	Normal cell doubling time; Viability >80%; at least two passages
10:90	0.5×10^6	Normal cell doubling time; Viability >80%; at least two passages
0:100	0.5×10^6	Adaptation complete when cells maintain normal doubling time; viability ≥90% over at least two passages

If media adaptation is not considered (**no media adaptation**), cells are grown and expanded using the original media and growth conditions. When the required cell number is reached, the cell suspension is used to inoculate the screening samples of the test medium. However, the carry-over volume of cells grown in original medium to the screening samples of the new medium should be limited. Sedimentation and resuspension of the amplified cells in the basal medium to be tested is an option to minimize media carryover (see 2.2).

For a first media screening, with the objective to quickly identify potential media candidates and feeding strategies out of many, direct media adaptation and no adaptation are common approaches for cell amplification. Having identified promising cell culture media and feeding strategies further trials are recommended for confirmation and optimization.

2.1 Direct media adaptation

Direct media adaptation means that the cell line is directly seeded and expanded in the basal test medium which is selected for media screening (Table 3). If EX-CELL® Advanced CHO is considered for media screening, then cells will be amplified in EX-CELL® Advanced CHO. If Cellvento® 4CHO is chosen for media screening, then cells will be expanded in Cellvento® 4CHO.

Table 3: Cell culture media for direct media adaptation and screening

Basal medium to be evaluated	EX-CELL® Advanced CHO	Cellvento® 4CHO
Medium for cell expansion	EX-CELL® Advanced CHO	Cellvento® 4CHO
Medium for screening	EX-CELL® Advanced CHO	Cellvento® 4CHO

Our standard protocol for direct media adaptation is provided below and the individual steps are demonstrated in Figure 1. Adaptation and cell expansion are performed at 30 mL scale. Direct media adaptation is started by inoculating the medium selected for cell expansion (see Table 3) directly from a cell bank vial or a culture actively growing in the original medium at a cell density of 0.5 x 10^6 cells/mL. An actively growing culture is considered for use when many cells need to be generated for the screening of different basal media but only one cell bank vial is available or should be consumed. Instead of starting direct media adaptation for each basal media to be screened with a cell bank vial, cell expansion is initiated with one cryovial using standard conditions and the original medium. After a few passages in original medium, when enough cells have been amplified, cells may be used to start the direct media adaptation cycle of the different basal media considered for screening. Pay close attention to carryover limits and limit the age of the cells (see protocol below).

Cells are passaged every 2–3 days at a cell density of 0.5 x 10^6 cells/mL. When a cell density of 1–3 x 10^6 cells/mL is reached and the viability is >90%, cells can be used as inoculum for the media screening trials.

Based on our standard protocol for direct media adaptation, a minimum total number of 30–90 x 10^6 cells should be expected in general in the final 30 mL culture after one expansion cycle. Higher cell densities may be achieved if the cells quickly adapt to the medium or experience a shorter doubling time. The key parameters for the execution of and the anticipated outcome from one cell expansion cycle are summarized in Table 4.

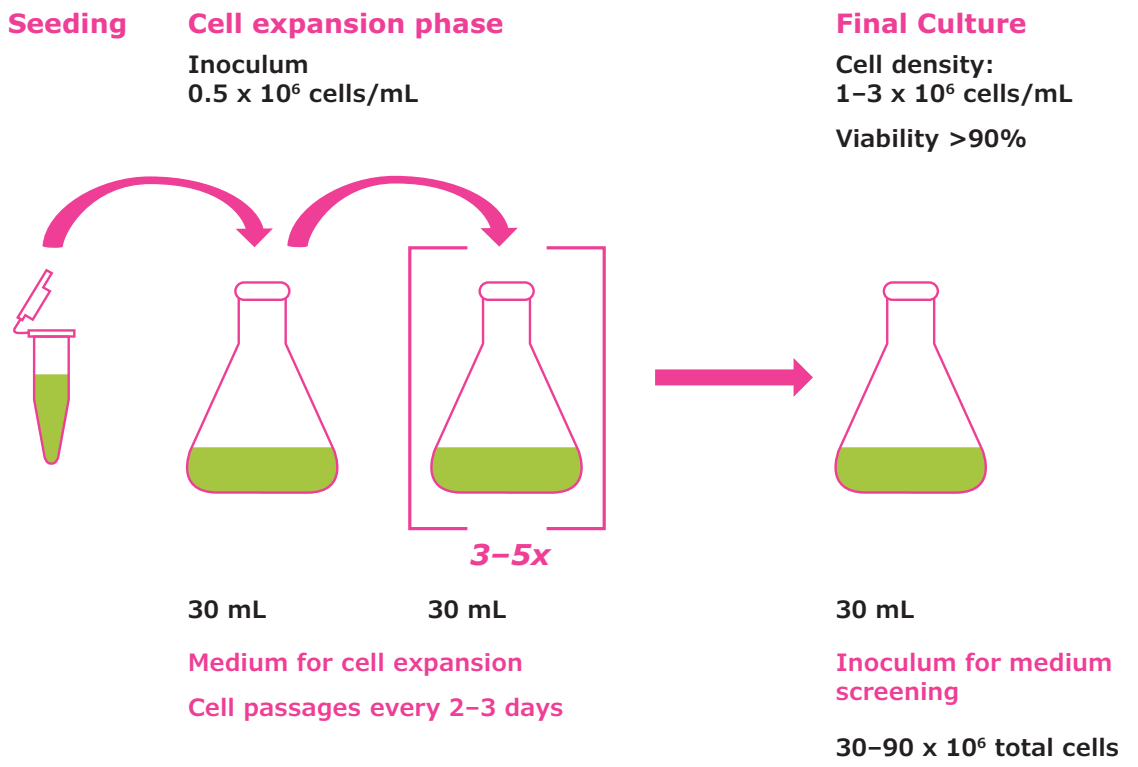
Be aware: Figure 1 and Table 4 are considered as reference for one cell expansion cycle of direct media adaptation. The estimates are calculated on average numbers based on experience and are for indication only. Every cell line has its own characteristics, and some cell lines may deviate from the average growth rates assumed here. So, higher or lower cell densities may be obtained depending on the properties of the cell line. This needs to be considered for the planning of the screening trials.

Table 4: Reference values for direct medium adaptation

Parameters for one cell expansion cycle of direct medium adaptation	
Seeding concentration (cells/mL)	0.5 x 10^6
Expansion to cell density (cells/mL)	1–3 x 10^6
Total cell number in 30 mL expansion culture	30–90 x 10^6
Viability at point of use	>90%
Cell passages every	2–3 days
No. of passages expected for cell expansion phase	3–5

The estimations for the expected total cell number reflect average values and are based on experience. They are considered for indication only. Depending on properties and growth behaviors of individual cell lines higher or lower cell densities may be possible after one expansion cycle.

Figure 1: Direct media adaptation—one cell expansion cycle



Protocol: Direct media adaptation and cell expansion

Cells are seeded in 30 mL medium for expansion at 0.5 x 10^6 cells/mL and then sub-cultured for at least 3 passages. When a cell density of 1–3 x 10^6 cells/mL and viability >90% has been reached the cells are ready for inoculating the screening samples (see Figure 1).

A) To initiate the cell culture from a cryovial, cells are thawed according to established laboratory instructions or the following procedure can be applied:

- Rapidly thaw (<1 minute) a vial of frozen cells in a 37 °C water bath.
- Transfer the CHO cell suspension from the cryovial aseptically into 30 mL of prewarmed cell culture medium selected for cell expansion (see Table 3).
- Incubate at 37 °C in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform (see Table 5).

B) To initiate cell expansion from an actively growing culture (meaning cells are grown under standard conditions using the original medium) carryover limits (see 2.2) and the age of the cell suspension should be considered. Cells should be from an early passage of a recent thaw.

- Spin down the cells grown in the current medium. Discard the supernatant and resuspend the cell pellet in the new cell culture medium selected for cell expansion. Resuspend in prewarmed medium at an appropriate cell density suitable for the inoculation.
- Inoculate the expansion samples at a cell density of 0.5 x 10^6 cells/mL with the cells from the resuspension up to a final volume of 30 mL.
- Incubate at 37 °C in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform (see Table 5).

Cell passaging is recommended at 2–3 days.

- Cell passage means the inoculation of fresh medium at a cell density of 0.5 x 10^6 cells/mL with the cells from the previous passage up to a final volume of 30 mL.
- However, carryover from one passage to the next one should not exceed a volume of 10 mL referring to a final working volume of 30 mL (≤33%). If more carry-over volume would be necessary, then this could be a sign that the cell line has difficulties to adapt to this specific medium and does not tolerate it well. One option could be to continue growing for a couple of additional passages to see if the adaptation reaches the targets. To respect carry-over volume in this case spin down the available/required cells and resuspend them in fresh medium. Alternatively, test the other basal medium option and proceed with the one that works best.
- A minimum of 3 passages should be performed. 3–5 passages are expected to reach appropriate cell densities and viabilities.
- Incubate at 37 °C in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform (see Table 5).

Cell culture splitting to increase total cells during direct media adaptation.

- If more cells are required for the screening trials than are expected to be generated by one direct medium adaptation cycle, splitting into additional or larger vessels could be an option to increase the final number of total cells. This means, after the 3rd or 4th passage, if cell density will allow more than one vessel with 30 mL fresh medium can be inoculated instead of only one. Or alternatively, a larger working volume may be selected for the next processing step.

Table 5: Process parameters for cell culturing at 30 mL scale

Vessel	Temperature (°C)	Humidity* (%)	CO_2 (%)	Speed (rpm)
125 mL Shake flask	37	80	5	70–80 (50 mm orbit)
125 mL Shake flask	37	80	5	100–110 (25 mm orbit)
50 mL Spin tube	37	80	5	230 (50 mm orbit)
50 mL Spin tube	37	80	5	320 (25 mm orbit)

*If humidity cannot be controlled at 80% general uncontrolled humidification should be utilized.

Equipment: Vessels with vented caps, working volume 30 mL

- TubeSpin® Bioreactors, 50 mL
- Erlenmeyer shake flask, 125 mL, ø26 mm

2.2 Cell expansion without media adaptation

Cells are amplified and grown under standard conditions which are usually applied in the lab for culturing the cell line in question. If the desired cell density and viability have been reached the cells are ready to be used as inoculum for the media screening samples.

However, the transfer of liquids from expansion cultures grown in original medium under standard lab conditions to the evaluation trials need to be reduced to the lowest level possible. The carryover should be no more than 1 mL for the inoculation of a 30 mL screening trial, this corresponds to ≤3% of the trial working volume. A cell density of ≥15 x 10^6 cells/mL would be required to stay below a carryover of 3%. These cell densities are not reasonable for use in continuous passaging.

Therefore, best practice is to spin down cells amplified in original medium and resuspend them in the new basal medium to be evaluated. In this case, the cells can be resuspended at an appropriate cell density suitable for the inoculation of the screening samples and carry-over limits can be neglected.

3. Cell culture media screening

This chapter is a reference tool for planning and performing media screening trials. It gives an overview on the different feeding possibilities and advises which options to evaluate. It also gives guidance on the number of screening samples that can be expected from 1 L liquid basal medium depending on the scale of the screening system and the execution of the screening trials.

3.1 Feeding strategy and feed mixing options

Two feeds are offered for CHO fed-batch processes: Cellvento® 4Feed and EX-CELL® Advanced Feed. They can be used as single feeds or mixed with each other at certain ratios.

Both feeds are compatible with the basal media Cellvento® 4CHO and EX-CELL® Advanced CHO offered for fed-batch processes (see Table 1 for media options).

It is recommended to test each basal medium with the individual feeds and with feed blends because it cannot be predicted which basal medium and which feeding option will work best for your cell line.

Table 6 gives an overview on the different feed mixing options that can be evaluated per basal medium. The feeds can be applied individually (100%) or as a mixture of certain ratios (mixing options no. 2–6).

Cellvento® 4Feed is a much higher concentrated feed than EX-CELL® Advanced feed. Therefore, the total amount of feed solution added to the cell culture over the study duration is 18% compared to 35% for EX-CELL® Advanced feed. For feed blends 30% is recommended (Table 6, Total feed addition).

The percentage of total feed is distributed across the run-time of the screening study and portions of feed are added to the culture vessel at certain days according to the dedicated feeding plan. This is shown in Table 6 and 7. Feeding plan A refers to the individual feed Cellvento® 4Feed, plan B to the feed blends and plan C to the individual feed EX-CELL® Advanced feed.

The percentage of feed to add at the day of feeding refers to the actual cell culture working volume in the culture vessel. In case an exact monitoring of volumes in and out of the culture vessel is not automated or cannot be reliably followed during small scale screening runs then the initial working volume can be taken as reference for the calculation of the feed volume.

The minimum feed options that are recommended to assess are the individual feeds and at least one feed mix. Based on our experience feed blends have the potential to outperform individual feeds and lead to higher growth rates and product titers. Therefore, at least 3 feeding options should be evaluated per basal media during an initial screening: the two individual feeds (mixing option 1 and 7 in Table 6) and the 50:50 ratio feed blend (mixing option 3, Table 6). This will give insight if individual feeds (and which one) or feed blends are the most promising feeding strategy for your cell line. A protocol for the screening of these minimum recommended options is provided in section 3.4.

Table 6: Feeding options per basal medium

Feed mixing options			Feeding strategy	
	Mixing ratio of			
No.	Cellvento® 4Feed	EX-CELL® Advanced Feed	Total feed addition (referring to culture vessel working volume)	Feeding plan applied
1	100%	0%	18%	A
2	75%	25%	30%	B
3	50%	50%	30%	B
4	25%	75%	30%	B
5	67%	33%	30%	B
6	33%	67%	30%	B
7	0%	100%	35%	C

Table 7: Feeding plans for feed mixing options in Table 6.

Feeding plan	A	B	C
Total feed addition per feeding plan	18%	30%	35%
Feeding schedule per feeding plan			
Feeding day	Feed distribution*	Feed distribution*	Feed distribution*
3	3%	5%	5%
5	3%	5%	5%
7	6%	7.5%	10%
10	3%	7.5%	7.5%
12	3%	5%	7.5%
Feed distribution*: The feed percentages per day are based on the working volume of the culture vessel at the time of feeding, not the initial volume.			
Glucose concentration in the culture vessel			
Feeding plan	A	B	C
Glucose conc.	4–8 g/L	4–8 g/L	4–8 g/L
	Monitor, maintain and adjust accordingly.		

Glucose should be monitored and added separately during feeding to maintain appropriate levels throughout the fed-batch culture. The individual feeds and feed mixtures do not contain glucose. 4–8 g/L are usually sufficient for the cells to span a 2-day period. Additional glucose may be needed by certain cells to withstand a 3-day period (i.e., feeding day 7–10). The actual glucose consumption depends on the specific energy requirements of the cells used for the media screening and should be considered for the culturing process.

In case of glutamine synthetase deficient cell lines (non-GS CHO cells), add 4–8 mM L-Glutamine (Cat. No. 59202C) to the basal medium prior use.

In case of dihydrofolate reductase deficient cell lines (non-DHFR CHO cells) add 1xHT (hypoxanthine/thymidine) supplement (Cat. No. H0137) prior use.

3.2 Screening trial samples and cell requirements

Common trial working volumes for cell culture media evaluation are 15 mL, 30 mL and 60 mL for shake flasks, Bioreactor-tubes (TubeSpin®) or Microbioreactor vessels (Ambr®) or 1–10 mL for plates. Media screening can be done in any system available with sufficient gas exchange.

For media screening, it is recommended to inoculate the trial samples at a cell density of 0.5×10^6 cells/mL. The total number of cells required to inoculate different sample working volumes is provided in Table 8.

As discussed in the previous section, at least 3 feeding options (2x individual feeds, 1x feed blend) should be evaluated per basal medium. If duplicates shall be performed, then 6 medium screening trials need to be executed per basal medium. The total number of cells required for the inoculation of 3 and 6 screening trial samples is also included in Table 8. The data in Table 8 should give an idea on how many cells need to be generated during cell expansion to enable the inoculation of the minimum recommended number of screening trials in relation to the trial working volume.

Table 8: Cell requirements for media screening working volumes

Total number of cells required for the inoculation 1, 3 and 6 screening trial samples related to the sample working volume			
Trial sample working volume (mL)	Total number of cells for		
	1x trial sample	3x trial samples	6x trial samples
1	0.5×10^6	1.5×10^6	3×10^6
10	5.0×10^6	15×10^6	30×10^6
15	7.5×10^6	22.5×10^6	45×10^6
30	15×10^6	45×10^6	90×10^6
60	30×10^6	90×10^6	180×10^6

Note: Cell density recommended for inoculation of the trial samples is 0.5×10^6 cells/mL

3.3 Number of screening samples related to 1 L liquid medium

This section will give some guidance on trial planning and provides estimates of how many screening samples can be expected from 1 L of liquid basal medium in relation to the sample working volume and whether direct media adaptation is applied or not.

3.3.1 Number of screening samples without media adaptation

When cells will not be adapted to the basal medium prior the actual media screening, then the entire volume of the 1 L liquid basal medium can be allocated to the screening trials. But be aware on carry-over limits when cells are grown and expanded using standard lab procedures and original cell culture media (see section 2.2).

If no basal cell culture medium needs to be reserved for cell adaptation, then the number of screening trials can be calculated based on the sample size and the maximum available volume of basal medium. This is shown in Table 9. For example, assuming a sample working volume of 15 mL for media screening, then 63 trial samples can be derived from 1 L liquid basal medium prepared from dry powder. The calculations are based on a final liquid volume of 950 mL considering potential medium losses during preparation and sterile filtration.

Table 9: Screening samples without applying media adaptation

Working Volume (mL) per screening sample	1	15	30	60
No. of trial samples from 1 L basal medium*	950	63	31	15

Note*: Calculations based on a final volume of 950 mL liquid medium to consider potential losses during medium preparation from dry powder (e.g., sterile filtration).

For an initial media screening study, a minimum number of 3 trials (2x individual feed, 1x feed blend) is recommended per basal medium (see section 3.1). This minimum number of screening trials can be performed for all scales listed in Table 9 including duplicates and triplicates from a 1 L cell culture medium sample if no volume needs to be allocated for the cell expansion step.

If a deeper evaluation of one of the basal media is considered and all seven feed mixing options described in Table 6 should be assessed to find the optimum growth conditions, then 1 L basal medium will also be sufficient for all working volumes in Table 9 to perform at least duplicate trials for all seven feed mixing options.

3.3.2 Number of screening trials including direct media adaptation

When cells are adapted to the cell culture medium before their use as an inoculum for the actual medium evaluation assay then a certain amount of liquid basal medium must be reserved for the cell adaptation steps.

In section 2.1, a protocol for direct media adaptation is provided and the different steps and process parameters are schematically described in Figure 1. According to this protocol, approximately 30 to 90 million cells are expected to be generated after one cell expansion cycle and about 150 mL basal medium are required for the execution of such a step including 5 passages at 30 mL. 30–90 x 10^6 total cells and a medium consumption of 150 mL will be the reference values for direct media adaptation referring to one cell expansion cycle. These values will be used for the calculations of the number of screening samples which can be derived from 1 L basal medium when media adaptation is considered.

The actual number of screening trials that can be performed is influenced by the following factors: the total number of cells generated during cell expansion, the amount of basal medium available for the screening study and the scale (working volume) of the screening trial samples.

The total number of cells required to inoculate one trial sample is defined by the working volume and the desired cell density for inoculation. A cell density of 0.5 x 10^6 cells/mL is recommended for the inoculation of the media screening samples. This means, that 0.5 x 10^6 cells will be required to inoculate one 1 mL screening sample, 7.5 x 10^6 cells to inoculate one 15 mL screening sample, 15 x 10^6 cells to inoculate one 30 mL screening sample and 30 x 10^6 cells to inoculate one 60 mL screening sample (Table 10).

Assuming a total number of 30 x 10^6 cells were generated during cell expansion and are available for the inoculation of the screening trials. If a working volume of 15 mL is considered per screening sample, then 4 trial samples can be inoculated at the desired cell density of 0.5 x 10^6 cells/mL. If 60 x 10^6 cells were produced during cell expansion then 8 screening trials of 15 mL could be performed, in case of 90 x 10^6 cells 12 screening trials of 15 mL can be executed (Table 10).

EX-CELL® Advanced CHO and Cellvento® 4CHO are the two basal media in Table 1 applied for fed-batch applications. Regarding these media, different feed options should be considered and tested during an initial media screening (section 3.1., Table 6). A minimum of 3 options is recommended per basal medium.

According to Table 10 for example, a total number of 30 x 10^6 cells allows the inoculation of 4 screening trials at a 15 mL working volume and therefore would enable the execution of the 3 recommended screening options. However, if all the 7 feed mixing options listed in Table 6 shall be evaluated for the basal medium of interest, to identify the optimum growth conditions, then the total number of 30 x 10^6 cells would not be sufficient.

Based on the scale of the working volume and the number of screening trials planned it must be checked how many cells will be required for the entire evaluation study and if one cell expansion cycle may be sufficient to produce enough cells to inoculate all screening samples.

Table 10 also provides information on the amount of medium which is necessary to perform the calculated number of trials per scale. For example, 60 mL basal medium is required to perform 60 trials at 1 mL or 4 trials at 15 mL or 2 trials at 30 mL or 1 trial at 60 mL. Assuming the cells for these trials are produced during one cell expansion cycle which consumes 150 mL of basal medium, then 150 mL medium needs to be added to the 60 mL to get the total volume of medium required to perform one of these trial sets including the cell expansion phase.

Table 10 is considered to provide reference values for the estimation of the number of screening trials based on the sample working volume and the total number of cells generated during one cell expansion cycle. Or the other way round, it indicates how many cells need to be produced to be able to perform a certain number of screening trials at a certain scale. The only constant parameter the calculations are based on is the cell density of 0.5 x 10^6 cells/mL recommended for the inoculation of the screening samples. The actual number of trials that ultimately can be performed varies with the scale of the trial working volume and the total cells generated for the inoculation of the screening samples.

The factual number of screening trials that can be derived from 1 L liquid basal medium using direct media adaptation greatly depends on the number of cells generated during one cell expansion cycle. This is shown in Table 11. For example, 4 screening trials are possible per cell expansion cycle of 30 x 10^6 cells (4/1) for 15 mL trial samples which will consume 210 mL of medium. This can be performed approximately 4x (= 840 mL) if about 1 L liquid basal medium is available. So, in terms of 15 mL sample working volume, approximately 16 screening trials are feasible based on 4 cell expansion cycles of 30 x 10^6 cells (16/4). One cell expansion cycle here is considered with the start of one cryovial as described in Figure 1. In reality, having to provide 4 cell bank vials to start 4 expansion cycles in parallel to generate enough cells for the screening trials is probably not a reasonable approach. Rather, one expansion cycle will be started from one cryovial and the cells are further propagated after a couple of passages by splitting (see section 2.1/protocol). In this case, less cell culture medium will be consumed for the cell amplification step compared to 4 full expansion cycles starting from a cell bank vial each. This could allow for even more screening trials to be achieved.

Table 10 and 11 are intended as reference for a first estimation of the number of screening trials which may be obtained from 1 L liquid basal medium in relation to the sample working volume and the total number of cells obtained from cell expansion as described in Figure 1. The calculations are based on average values observed for standard CHO clones in suspension and cell culture procedures applied in our lab.

Table 10: Estimations of screening trials and media consumption

Number of screening trials related to total cells generated during one cell expansion cycle						
Trial sample working volume (mL)	1	15	30	60	**Media requirements for number of trials**	
Number of cells per working volume*	0.5 x 10^6	7.5 x 10^6	15 x 10^6	30 x 10^6		
Total number of cells**	**Number of trials per total cells**				**(mL)**	Including Cell expansion*** (mL)
30 x 10^6	60	4	2	1	**60**	210
60 x 10^6	120	8	4	2	**120**	270
90 x 10^6	180	12	6	3	**180**	330

*Recommended seeding density = 0.5 x 10^6 cells/mL

**Generated during one cell expansion cycle of 5 passages requiring 150 mL basal medium (Fig.1)

*** Adding 150 mL for one cell expansion cycle of direct media adaptation to the medium volume required for the number of trials per scale (working volume).

Table 11: Estimations of media screening trials from 1 L medium

Number of screening samples per 1 L basal medium including direct medium adaptation						
Trial sample working volume (mL)		1	15	30	60	**Medium requirements (mL)**
Total number of cells per cell expansion cycle		**Number of trials per cell expansion and working volume** (No. of trial samples / No. of expansion cycles)				
30 x 10^6		60/1	4/1	2/1	1/1	**210**
	max.	240/4	16/4	8/4	4/4	**840**
60 x 10^6		120/1	8/1	4/1	2/1	**270**
	max.	360/3	24/3	12/3	6/3	**810**
90 x 10^6		180/1	12/1	6/1	3/1	**330**
	max.	450/2.5	30/2.5	15/2.5	7/2.5	**825**

Be aware, if direct media adaptation is applied for cell expansion the carry-over volume to inoculate the screening sample should be ≤25% of the working volume. This means, cell densities ≥2 x 10^6 cells/mL in the cell expansion culture at the time point of harvest are required for a direct inoculation of the screening trials. Otherwise, cell sedimentation and resuspension in fresh medium at the desired cell density is recommended.

3.4 Execution of screening trials

A protocol for the screening of our fed-batch basal media and the different feeding options is provided here.

As described in section 3.1, two feeds (Cellvento® 4Feed and EX-CELL® Advanced Feed) are available for feeding and can be applied individually or as mixture. To identify the best feeding option the two feeds shall be evaluated individually and as blends per basal medium considered for screening.

Table 12 covers the minimum feed options including their feeding plan and schedule which are recommended to be tested per basal medium during an initial media screening. These are the two individual feeds (100%) and a feed mix of a 50:50 ratio to gain insight if individual feeds or feed blends may work best for your cell line.

The three minimum feeding options shall be evaluated for the basal media Cellvento® 4CHO and EX-CELL® Advanced CHO because it cannot be predicted upfront which basal medium will suit your cell line better and enable faster growth, higher cell densities and product titers. If utilizing direct media adaptation, you may come to know, prior to the screening trials, if there is a basal medium not well tolerated by your cell line.

If the basal medium has been selected, then it may be worth to evaluate more than the minimum three feed options mentioned above to identify the optimum growth conditions for your cell line. All feed blends follow the feeding plan B, so the column “Feed mix” in Table 12 is applicable to all feed mixing ratios.

Be aware, if no blending occurs and the two individual feeds are supplemented as pure solutions (100%) then different feeding plans apply. This is feeding plan A for Cellvento® 4Feed and feeding plan C for EX-CELL® Advanced Feed.

The difference in the feeding plans lies in the maximum feed added during the screening study to the cell culture (Table 12, total feed addition). This means that the percentage of feed volume added at the day of feeding to the cell culture differs between the different feeding plans (feeding schedule). The feed percentages are based on the working volume in the culture vessel at the time of feeding, not the initial volume. In the case of an automated system the input and output of the cell culture are automatically monitored, and the actual working volume is provided to calculate the actual feed volume. In case of small screening trials if exact monitoring of input and output to the cell culture is not feasible, the initial volume can be used to calculate the feed volume at the day of feeding.

Table 12: Screening trial plan to be evaluated per basal medium

Feed option	Individual Feed 1	Feed mix	Individual Feed 2
Feed	Cellvento® 4Feed	Cellvento® 4Feed and EX-CELL® Advanced Feed	EX-CELL® Advanced Feed
Mixing ratio	100%	50% : 50%	100%
Feeding plan	**A**	**B**	**C**
Total feed addition (%)	18	30	35
Feeding schedule			
Feeding day	Feed distribution*	Feed distribution*	Feed distribution*
3	3%	5%	5%
5	3%	5%	5%
7	6%	7.5%	10%
10	3%	7.5%	7.5%
12	3%	5%	7.5%
Feed distribution*: The feed percentages per day are based on the working volume of the culture vessel at the time of feeding, not the initial volume.			
Screening trial operational parameters and remarks			
Inoculation density (cells/mL)		0.5×10^6	
End of assay		Day 14 or if cell viability decreases to <70%	
Determine cell density and viability		At day of feeding and day 14	
Determine product titer at day		7, 10, 12, 14	
Parameters recommended to monitor		Main: pH, glucose, lactate, ammonia Optional: sodium, potassium, glutamine, glutamate, calcium Measure at day of feeding and day 14	
Temperature		37 °C	
Humidity		70–80% if controllable	
Supplements			
Glucose		4–8 g/L monitor, maintain and adjust accordingly	
Selection system additives (if required)			
Selection marker: Glutamine synthetase (GS)		In case of glutamine synthetase deficient cell lines, add 4–8 mM L-Glutamine (Cat. No. 59202C) to the basal medium prior use.	
Selection marker: Dihydrofolate reductase (DHFR)		In case of dihydrofolate reductase deficient cell lines add 1xHT (hypoxanthine/thymidine) supplement (Cat. No. H0137) prior use.	

An overview on the number of cells which are required to inoculate the individual working volumes and three or six samples are provided in Table 8, section 3.2. The calculations are based on the recommended cell density of 0.5×10^6 cells/mL for inoculating the screening samples. One must make sure that enough cells are generated during cell expansion phase to be able to inoculate all the desired screening samples at the recommended cell density (see chapter 2).

4. Media preparation

4.1 Basal media hydration

Basal medium: EX-CELL® Advanced CHO (Cat. 24366C)

Step	Action	Supplement/Parameter	Amount / Specification	Comments
1	Add	Water (RT)	0.8 L	RT = 25–40 °C
2	Add	CCM powder	22.09 g	Add slowly while stirring
3	Stir	vigorously for	15 min	Fluid will remain slightly turbid
4	Adjust	pH	5.0	Adjust with 5 N NaOH
5	Add	Sodium bicarbonate	1.9 g	–
6	Stir	at least for	30 min	Fluid should become clear
7	Adjust	pH	7.2 ±0.1	Adjust with 5 N NaOH
8	Add	Water (RT)	up to 1 L	Add to final volume
Final volume			**1 L**	
9	Measure	Osmolality	280–320 mOsmo/kg	Confirm specification
10	Sterilizing filtration right after preparation			

Remarks	
Application	Amplification and production medium for fed-batch applications.
Feed media	Can be combined with the feeds Cellvento® 4Feed, EX-CELL® Advanced Feed or a mix of both.
Notes	This medium does NOT contain L-glutamine, or hypoxanthine and thymidine.
	During a fed-batch process glucose needs to be added and maintained according to the energy requirements of your cell. General recommendations: 4–8 g/L glucose.
	Use Milli-Q® or similar cell culture grade water for media hydration.
Storage	Store media dry powder at 2–8 °C protected from light.
	Store reconstituted liquid medium at 2–8 °C protected from light.
Shelf life	Dry powder is stable for 36 months at 2–8 °C. Respect expiration date.
	Reconstituted liquid medium is stable for 180 days after preparation (unopened) and stable for 30 days AFTER opening.
	When supplements are added, the liquid medium is stable for max. 4 weeks.
Comment	Refer to general rules for dry powder hydration.

Basal medium: Cellvento® 4CHO (Cat. 103795)

Step	Action	Supplement/Parameter	Amount / Specification	Comments
1	Add	Water (RT)	0.8 L	RT = 25 °C will be necessary
2	Add	CCM powder	23.7 g	Add slowly while stirring
3	Stir	Vigorously	30 min	Fluid will remain slightly turbid
4	Add	Sodium bicarbonate	2 g	while stirring
6	Stir	for	~10 min	Fluid should become clear
7	Add	Water (RT)	up to 1 L	Add to final volume
Final volume			**1 L**	
8	Measure	pH	7.0 ±0.3	Confirm pH specification
9	Measure	Osmolality	310 ±30 mOsmo/kg	Confirm specification
10	Sterilizing filtration right after preparation			

Remarks	
Application	Amplification and production medium for fed-batch applications.
Feed media	Can be combined with the feeds Cellvento® 4Feed, EX-CELL® Advanced Feed or a mix of both.
Notes	This medium does NOT contain L-glutamine, or hypoxanthine and thymidine.
	During a fed-batch process glucose needs to be added and maintained according to the energy requirements of your cell. General recommendations: 4–8 g/L glucose.
	Use Milli-Q® or similar cell culture grade water for media hydration.
Storage	Store media dry powder at 2–8 °C protected from light.
	Store reconstituted liquid medium at 2–8 °C protected from light.
Shelf life	Dry powder is stable for 18 months at 2–8 °C. Respect expiration date.
	Reconstituted liquid Cellvento® 4CHO medium is stable for at least 90 days.
	When supplements are added, the liquid medium is stable for max. 4 weeks.
Comment	Refer to general rules for dry powder hydration.

4.2 Feed media hydration

Feed medium: EX-CELL® Advanced Feed (Cat. 24368C)

Step	Action	Supplement/Parameter	Amount/Specification	Comments
1	Add	Water (RT)	0.8 L	RT
2	Add	CCM powder	34.1 g	Add slowly while stirring
3	Stir	Vigorously	30 min	Fluid will remain slightly turbid
4	Adjust	pH	9.5 ±0.1	Adjust with 5 N NaOH
5	Stir	for	10 min	Fluid should become clear
6	Adjust	pH	8.5 ±0.3	Adjust with 5 N HCl
7	Stir	for	10 min	
8	Add	Water (RT)	up to 1 L	Add to final volume
Final volume			**1 L**	
9	Measure	Osmolality	450–550 mOsmo/kg	Confirm specification
10	Sterilizing filtration right after preparation			

Remarks	
Application	Feed medium for fed-batch applications.
Usage	Can be used as single feed or in combination with Cellvento® 4Feed.
	Applicable for basal media EX-CELL® Advanced CHO and Cellvento® 4CHO.
Notes	During a fed-batch process glucose needs to be added and maintained according to the energy requirements of your cell. General recommendations: 4–8 g/L glucose.
	Use Milli-Q® or similar cell culture grade water for media hydration.
Storage	Store media dry powder at 2–8 °C protected from light.
	Store reconstituted liquid medium at 2–8 °C protected from light.
Shelf life	Dry powder is stable for 36 months at 2–8 °C. Respect expiration date.
	Reconstituted liquid medium is stable for 1 month.
Comment	Refer to general rules for dry powder hydration.

Feed medium: Cellvento® 4Feed comp (Cat. 103796)

Step	Action	Supplement/Parameter	Amount/Specification	Comments
1	Add	Water (RT)	0.9 L	RT
2	Add	CCM powder	130.35 g	Add slowly while stirring
3	Stir	vigorously for	45 min	Until fully dissolved
4	Adjust	pH	7.0 ±0.3	Adjust slowly with 5 N NaOH
5	Add	Water (RT)	up to 1 L	Add to final volume
Final volume			**1 L**	
6	Measure	pH	7.0 ±0.3	Confirm pH specification
7	Measure	Osmolality	1220 ±50 mOsmo/kg	Confirm specification
8	Sterilizing filtration right after preparation			

Remarks	
Application	Feed medium for fed-batch applications.
Usage	Can be used as single feed or in combination with EX-CELL® Advanced Feed.
	Applicable for basal media EX-CELL® Advanced CHO and Cellvento® 4CHO.
Notes	During a fed-batch glucose needs to be added and maintained according to the energy requirements of your cell. General recommendations: 4–8 g/L glucose.
	Use Milli-Q® or similar cell culture grade water for media hydration.
Storage	Store media dry powder at 2–8 °C protected from light.
	Store reconstituted liquid medium at 2–8 °C protected from light.
Shelf life	Dry powder is stable for 24 months at 2–8 °C. Respect expiration date.
	Reconstituted liquid medium is stable for 60 days.
	When a bottle is opened, the liquid medium is stable for max. 3 weeks.
Comment	Refer to general rules for dry powder hydration.

4.3 Addition of supplements

The media mentioned in this protocol are designed to be applicable for a broad range of CHO cell lines and selection systems.

The basal media and feeds mentioned represent complete media which in general do not need additional supplementation for feeding except of glucose. The feeds do not contain glucose, as such glucose should be added during a fed-batch process according to the energy requirements of your cell line. The glucose concentration should be monitored, maintained, and adjusted at the required level during feeding.

Additional supplements may be required depending on cell specific needs. If a selection system such as glutamine synthetase (GS) or dihydrofolate reductase (DHFR) is used, then the production media need to be supplemented with the corresponding components. This means the addition of 4–8 mM L-Glutamine (Cat. No. 59202C) in case of a GS deficient cell line or 1xHT (hypoxanthine/thymidine; Cat. No. H0137) in case of a cell line lacking DHFR functionality. The basal media do not contain glutamine, or hypoxanthine and thymidine.

4.4 Feed mixing guidelines

As cell lines can be inherently diverse, it can be recommended when evaluating feeds to try several different mix levels to find the optimal level of feed for your process (see section 3.1).

We recommend evaluating Cellvento® 4Feed and EX-CELL® Advanced Feed both as standalone feeds and as a mix (see section 3.1).

The following points should be considered for the preparation of feed mixes:

1. The powders cannot be mixed!
2. Hydrate each feed powder separately according to their individual protocol as described in section 4.2.
3. The two feeds can be mixed when they are in their liquid format and fed as one feed (= one feed approach).
4. The liquid feeds can be combined and sterile filtered as one liquid. Or sterile feeds can be mixed at the desired ratio as long as sterility is maintained.
5. Combined feed mixes of all ratios are considered stable for 4 weeks.
6. Each feed can also be fed separately as long as the overall ratios are maintained (= two feed approach).

   Example: A 50:50 feed ratio requires a 5% total feed at day 3 → meaning 2.5% of Cellvento® 4Feed and 2.5% of EX-CELL® Advanced Feed are added separately to the cell culture.

Preparation of feed mixes:

1. Add reconstituted liquid feed media (Cellvento® 4Feed and EX-CELL® Advanced Feed) at the desired ratio to a mixer.
2. Mix together for 10 minutes until homogenous.
3. Store at 2–8 °C protected from light until use.
4. Discard any unused feed mix after one month.

4.3 Sterile filtration

Cell culture media should be aseptically filtered through a 0.2 µm or 0.1 µm sterilizing-grade membrane filter right after preparation in order to prevent bacterial contamination and growth. Generally, 0.2 µm membrane filters are commonly applied for bioburden reduction and sterilizing filtration but in case of an increased assurance against Mycoplasma contamination 0.1 µm sterilizing-grade membranes are selected.

On laboratory scale and under non-GMP conditions as for example in research and early developments labs, vacuum-driven filter devices such as Stericup® QR/Steritop® QR or the new eco-friendly Stericup® E/Steritop® E are commonly used for bioburden reduction of cell culture media volumes between 150 mL–1 L.

https://www.sigmaaldrich.com/products/cell-culture-and-analysis/sterile-lab-media-filtration-and-cultureware

https://www.sigmaaldrich.com/products/cell-culture-and-analysis/sterile-lab-media-filtration-and-cultureware/stericup-vacuum-filtration

For GMP production processes and larger process volumes different filter devices are required for bioburden reduction or sterilizing filtration. Filter capsules (Opticap®) of various sizes are available to suit different process scales. Filter capsules are offered with different types of sterilizing grade membranes (Durapore® (PVDF), Millipore Express® (PES)) and pore sizes applicable for various sterilizing filtration applications and volumes.

Application Note "Cell Culture Media Filtration: Filter Selection and Sizing" (Lit. No. MS_AN5144EN Ver. 1.0, 4/2020)

Millipore® Application Note: Cell Culture Media Filtration Evaluating Cell Culture Performance (Lit. No. MS_AN7112EN Ver. 1.0)

For technical assistance on filter selection refer to the corresponding media data sheet or contact: **EMDMillipore.com/contactPS**

Technical Assistance

For more information, please visit **SigmaAldrich.com** for up-to-date worldwide contact information

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