Instructions for Use

4Cell® SmartCHO Media System

A Chemically Defined and Animal Component Free Cell Culture Media for any CHO Cell Lines

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SARTORIUS

Contents

1 Introduction

4Cell® SmartCHO Media System consists of four different media:

- 4Cell® SmartCHO Stock & Adaptation Medium (SAM)
- 4Cell® SmartCHO Production Medium (PM)
- 4Cell® SmartCHO Feed Medium A (FMA)
- 4Cell® SmartCHO Feed Medium B (FMB)

These media are chemically defined non-animal origin growth media formulated to maximize the product titer in CHO cell lines. It provides robust performance in production systems of small and large scale, e.g. flask, Ambr® 15 | 250 and bioreactors.

Product Characteristics:

- Complete medium
- Animal Component Free (ACF) medium
- Serum-free (SF) medium
- Does not require the addition of serum.
- Does not contain antibiotics.
- The medium requires the addition of Glutamine.
- Available in as non-sterile powder and as sterile liquid.

1.1 Intended Use and Safety Statements

For the intended use, please refer to Certificate of Analysis.
Not approved for human or veterinary use. Not for application in humans or animals, or for use in vitro diagnostic or clinical procedures.

1.2 Stability

4Cell® SmartCHO Stock & Adaptation Medium (SAM) and 4Cell® SmartCHO Production Medium (PM) need to be supplemented with L-Glutamine prior to use following the instructions for use, usually at 4 – 6 mM final concentration. After addition of L-Glutamine, the media are stable for 4 weeks when stored at 2 – 8°C protected from light.

Both feeds, 4Cell® SmartCHO Feed Medium A (FMA) and 4Cell® SmartCHO Feed Medium B (FMB), are ready for use and do not require L-Glutamine supplementation.

1.3 Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. When not in use store 4Cell® SmartCHO Media System components at 2°C to 8°C protected from light.

1.4 Recommended Materials

- DMSO for cell preservation
- 125 mL Erlenmeyer flask, Ambr® 15 | 250, 2 L – 50 L bioreactors
- 100 – 400 g/L sterile filtered Glucose
- L-Glutamine, 200 mM
- PES membrane filter with 0.2 µm pore size, e.g. Sartopore 2® (5441307H5--OO--B)

2 Instructions for Use

2.1 Adapting Cell Lines to 4Cell® SmartCHO Medium

In general, there are two approaches to adapt a cell line to a new culture medium formulation.

- **Option 1:** Passage directly the culture directly from the initial medium into 4Cell® SmartCHO Stock & Adaptation Medium (SAM). Choose a high seeding cell density at each passage (e.g. 5 x 10^5 cells/mL) for a minimum of two weeks.
  When the cells achieve a stable growth rate and viability >90% for 2 passages, the adaptation is considered complete.
- **Option 2:** Passage the culture into a mixture of original culture medium and 4Cell® SmartCHO SAM and gradually increase the content of 4Cell® SmartCHO SAM. An example for a step-wise adaptation protocol is given below.

Adaptation step	Ratio of original medium to 4Cell® SmartCHO SAM	Acceptance criterion to proceed to next adaptation step
1	75:25	Viability ≥90% of original medium normal doubling time for 2 passages
2	50:50	Viability ≥90% of original medium normal doubling time for 2 passages
3	25:75	Viability ≥90% of original medium normal doubling time for 2 passages
4	0:100	Adaptation complete if viability >90% in 4Cell® SmartCHO SAM medium normal doubling time for 2 passages

2.2 Cell Cultivation

- Cultivate the cells in an incubator with a shaking platform and humidified atmosphere, containing 7.5% ± 0.5% CO_2.
- Other cultivation parameters may be adapted to each cell line's individual requirements. A recommended starting point is a temperature of 36.8°C ± 0.2°C and 103 rpm* on an orbital shaking platform.
- By regular passaging of the cells, ensure that the culture remains in mid- exponential growth phase at all times. Determine cell density and viability of the culture every 2 to 3 days and dilute the culture to a suitable seeding density with fresh pre-warmed medium (e.g. 3 x 10^5 viable cells/mL).

2.3 Thawing of Cells | Initiation of Culture Process

1. Pre-warm 4Cell® SmartCHO Stock & Adaptation Medium (SAM) to cultivation temperature before use. The required medium volume depends on the cell density in frozen cryovials.
   The cell density after thawing should be 3 – 5 x 10^5 viable cells/mL.
2. After removing cryovial from storage, wipe the cryovial with 70% v/v ethanol or isopropanol before opening. In a Biological Safety Cabinet (BSC), briefly twist the cap a quarter turn to relieve pressure, and then retighten.
3. Quickly thaw the cryovial in a 37°C water bath (do not submerge the cryovial completely) or heating block at 37°C until only a small grain of ice remains. Thawing the cells for longer than 3 minutes may result in reduced cell viability.
4. Dry the cryovial with a lint-free wipe, spray with 70% v/v ethanol or isopropanol, and then wipe to remove excess liquid.
5. Immediately transfer the thawed cell suspension with a pipette into 10 mL of 4Cell® SmartCHO SAM and centrifuge at 180 – 200 x g for 3 minutes. Remove the supernatant carefully.
6. Carefully reconstitute the cell pellet in fresh pre-warmed 4Cell® SmartCHO SAM by gently mixing by pipetting up and down.
7. Transfer the suspension as inoculum into the culture vessel. Proceed with cell cultivation as described above.

* `shaking rate for an Infors Multitron cell incubator with 50 mm orbital diameter. For shakers with other orbital diameters: shaking rate in rpm = 103 x (50/orbital diameter)$^{1/2}$

2.4 Freezing of Cells | Storage

The cell culture should be in mid-logarithmic growth phase and >90% viable at the point of freezing.

1. Prepare the necessary volume of freezing medium by supplementing 4Cell® SmartCHO Stock & Adaptation Medium (SAM) with 7.5% Dimethylsulfoxide (DMSO). L-Glutamine can be added optionally (4 – 6 mM final concentration) to the freezing medium, but is not necessary. Store the freezing medium at 2 – 8°C until use.
2. Transfer the required volume of cell suspension into centrifugation vessels and spin down the cells at 180 – 200 x g for 5 minutes. Gently remove the supernatant.
3. Reconstitute the cell pellet in the required volume of freezing medium to achieve a cell density of at least 1 x 10^7 viable cells/mL. Dispense the suspension into cryovials, taking care that the suspension remains homogenous at all times.
4. Use a suitable controlled cooling method to freeze the vials, ideally a controlled-rate freezer. Alternatively, place them in a cell freezing container overnight at –80°C. For storage keep the vials at a temperature below –130°C preferably in vapor phase LN2 for frequent access and in liquid nitrogen freezer for long-term storage.

2.5 Protein Production in Fed-Batch Mode

The 4Cell® SmartCHO Media System Starting Kit includes all necessary medium components for adaptation of CHO cell lines, cultivation from stock culture of adapted cells up to 3 L of fed-batch cultivation in 4Cell® SmartCHO Production Medium (PM) plus Feed Medium A (FMA) and Feed Medium B (FMB). Stirred bioreactors and Sartorius Ambr® 15 | 250 are ideally suited to carry out comprehensive evaluation of fed-batch parameters in the course of process development.

The Table 1 below outlines volumes needed for each individual medium component dependent on the target cultivation volume from seed train to the end of fed-batch culture.

NOTE
The actual volume needed for adaptation to 4Cell® SmartCHO Stock & Adaptation Medium (SAM) is dependent on the cell type and adaptation method used.

Culti-vation Volume	Liquid Volumes				Culti-vation Volume	Liquid Volumes			
	SAM	PM	FMA	FMB		SAM	PM	FMA	FMB
L	L	L	L	L	L	L	L	L	L
2	0.52	1.20	0.60	0.08	5	1.3	3	1.5	0.2
2.5	0.65	1.50	0.75	0.10	10	2.6	6	3	0.4
3	0.78	1.80	0.90	0.12	15	3.9	9	4.5	0.6
4	1.04	2.40	1.20	0.16	20	5.2	12	6	0.8

Table 1

2.6 Fed-Batch Protocol

The following protocol in best carried out in a stirred tank bioreactor. Alternatively, Ambr® 15 | 250 systems or shake flasks can be used.

After successful adaptation, seed the cells in the desired volume of completed 4Cell® SmartCHO Production Medium (PM) at a suitable viable cell density (recommended: 3×10^5 cells/mL). If the volume of inoculum is less than 20% of the final volume, cells can be transferred directly from 4Cell® SmartCHO SAM to 4Cell® SmartCHO PM; otherwise it is recommended to centrifuge the cells to remove the used 4Cell® SmartCHO SAM and reconstitute them in fresh 4Cell® SmartCHO PM.

From day 3 after inoculation, perform daily sampling and monitoring of cell density, viability, product titer and key metabolites.

Begin the addition of 4Cell® SmartCHO Feed Medium A (FMA) and 4Cell® SmartCHO Feed Medium B (FMB) on day 3 after inoculation, or when the viable cell density reaches 2×10^6 cells/mL. Suggested feeding options for a fed-batch are outlined in the Tables 2a – b below. Other cultivation conditions are the same as outlined above in paragraph "Cell cultivation".

Add 4Cell® SmartCHO FMA at 2 – 4% of original culture volume and 4Cell® SmartCHO FMB at 10% of 4Cell® SmartCHO FMA volume. Add both feed media slowly and ensure that the feed media are quickly dispersed within the cultured cells. FMB has a pH of 10, so its addition may lead to a short spike in culture pH.

4Cell® SmartCHO FMA contains sufficient glucose to supply the fed-batch culture until day 5 after inoculation. Glucose concentration should be measured daily and kept at a value of 4 – 6 g/L by addition of a separate concentrated solution (recommended concentration: 400 g/L) when required.

End the cultivation when a pre-determined end criterion is reached, e.g. a specific time point after inoculation or when viability falls below 50 – 70%.

Table 2: Two suggested options for a fed-batch procedure to determine the optimal daily feeding rate. The ratio of FMA to FMB should always be 10:1. Values are calculated as percentages of starting values at day 0.

Day	0	1	2	3	4	5	6	7	8	9
FMA	–	–	–	2%	2%	2%	2%	2%	2%	2%
FMB	–	–	–	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Glucose	–	–	–	–	–	To target concentration, 4 – 6 g/L				

Day	10	11	12	13
FMA	2%	2%	2%	2%
FMB	0.2%	0.2%	0.2%	0.2%
Glucose	To target concentration, 4 – 6 g/L			

Table 2a) Option A: 2% feeding strategy

Day	0	1	2	3	4	5	6	7	8	9
FMA	–	–	–	4%	4%	4%	4%	4%	4%	4%
FMB	–	–	–	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%
Glucose	–	–	–	–	–	To target concentration, 4 – 6 g/L				

Day	10	11	12	13
FMA	4%	4%	4%	4%
FMB	0.4%	0.4%	0.4%	0.4%
Glucose	To target concentration, 4 – 6 g/L			

Table 2b) Option B: 4% feeding strategy

IMPORTANT

For cells depending on continuous supply with L-Glutamine the cultures may need to be supplemented with additional L-Glutamine to prevent its depletion.

3 Instructions for Reconstitution of 4Cell® SmartCHO Media Powder (Optional)

The media is packaged and intended to be hydrated completely from one container.

3.1 4Cell® SmartCHO Stock & Adaptation Medium

1. Fill WFI (Water for Injection) into the appropriate mixing vessel. The WFI should be at room temperature. To allow pH adjustment later, the volume should be 95% of the final volume.
2. Add 20.04 g/L of the media powder 4Cell® SmartCHO Stock & Adaptation Medium and stir rapidly for a minimum of 30 min, or until no powder clumps remain. Choose a stirring speed high enough to quickly draw the powder under the surface, but low enough to avoid air bubbles and foaming.
3. Without suspending stirring, stepwise add 1.1 mL of 5 M NaOH solution or 0.55 mL 10 M NaOH solution per liter medium and continue to stir for a minimum of 30 min, or until all powder is dissolved.
4. Add 1.80 g/L $NaHCO_3$ and stir until completely dissolved (~15 min).
5. If required, adjust the pH to 6.90 – 7.35 by adding 5 M or 10 M NaOH
6. Add WFI to the final volume and stir for 20 minutes. Note that longer stirring times after the addition of $NaHCO_3$ will lead to a gradual increase in pH. The osmolality value of the liquefied 4Cell® SmartCHO Stock & Adaptation Medium is expected to stand within 270 – 330 mOsmol/kg H_2O.
7. Sterile filter the medium using a PES membrane filter with 0.2 µm or 0.1 µm pore size. Using 0.1 µm pore size ensures the removal of mycoplasma in addition to other microorganisms.

Store at 2 – 8°C. Protect from light.

3.2 4Cell® SmartCHO Production Medium

1. Fill WFI (Water for Injection) into the appropriate mixing vessel. The WFI should be at room temperature. To allow pH adjustment later, the volume should be 95% of the final volume.
2. Add 22.34 g/L of the media powder 4Cell® SmartCHO Production Medium and stir rapidly for a minimum of 30 min, or until no powder clumps remain. Choose a stirring speed high enough to quickly draw the powder under the surface, but low enough to avoid air bubbles and foaming.
3. Without suspending stirring, stepwise add 5.5 mL of 5 M NaOH solution, or 2.75 mL of 10 M NaOH solution, per liter medium and continue to stir for a minimum of 30 min, or until all powder is dissolved.
4. Add 1.80 g/L $NaHCO_3$ and stir until completely dissolved (~15 min).
5. If required, adjust the pH to 6.90 – 7.35 by adding 5 M or 10 M NaOH.
6. Add WFI to the final volume and stir for 20 minutes. Note that longer stirring times after the addition of $NaHCO_3$ will lead to a gradual increase in pH. The osmolality value of the liquefied 4Cell® SmartCHO Production Medium is expected to stand within 280 – 340 mOsmol/kg H_2O.
7. Sterile filter the medium using a PES membrane filter with 0.2 µm or 0.1 µm pore size. Using 0.1 µm pore size ensures the removal of mycoplasma in addition to other microorganisms.

Store at 2 – 8°C. Protect from light.

3.3 4Cell® SmartCHO Feed Medium A

1. Fill WFI (Water for Injection) into the appropriate mixing vessel. The WFI should be at room temperature. To allow pH adjustment later, the volume should be 85% of the final volume.
2. Add 168.78 g/L of the media powder 4Cell® SmartCHO Feed Medium A and stir rapidly for a minimum of 30 min, or until no powder clumps remain. Choose a stirring speed high enough to quickly draw the powder under the surface, but low enough to avoid air bubbles and foaming.
3. Without suspending stirring, add 13 mL of 5 M NaOH solution or 6.5 mL of 10 M NaOH solution per liter medium and continue to stir for a minimum of 60 min, or until powder is completely dissolved.
4. If required, adjust the pH to 6.50 – 6.80 by adding 5 M or 10 M NaOH.
5. Add WFI to the final volume and stir for 20 minutes. The osmolality value range of the liquefied 4Cell® SmartCHO Feed Medium A is expected to stand within 233 – 293 mOsmol/kg H_2O, measured at a 1:5 dilution.
6. Sterile filter the medium using a PES membrane filter with 0.2 µm or 0.1 µm pore size. Using 0.1 µm pore size ensures the removal of mycoplasma in addition to other microorganisms.

Store at 2 – 8°C. Protect from light.

3.4 4Cell® SmartCHO Feed Medium B

1. Fill WFI (Water for Injection) into the appropriate mixing vessel. The WFI should be at room temperature. To allow for pH adjustment later, the volume should be 75% of the final volume.
2. Add 110.71 g/L of the media powder 4Cell® SmartCHO Feed Medium B and stir rapidly for a minimum of 30 min, or until no powder clumps remain. Choose a stirring speed high enough to quickly draw the powder under the surface, but low enough to avoid air bubbles and foaming. The solution will remain cloudy in this step.
3. Without suspending stirring, stepwise add 140 mL 5M NaOH or 70 mL 10 M NaOH solution per liter medium and continue to stir for a minimum of 60 min at room temperature. The solution must be clear, and all powder dissolved at the end of this step.
4. If required, adjust the pH to 10.4 – 10.6 by adding 5 M or 10 M NaOH.
5. Add WFI to the final volume and stir for 20 minutes. The osmolality value range of the liquefied 4Cell® SmartCHO Feed Medium B is expected to stand within 185 – 225 mOsmol/kg H_2O, measured at a 1:5 dilution.
6. Sterile filter the medium using a PES membrane filter with 0.2 µm 0.1 µm pore size. Using 0.1 µm pore size ensures the removal of mycoplasma in addition to other microorganisms.

Store at 2 – 8°C. Protect from light.

4 Contacts

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

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