Instructions for Use

4Cell® SmartCHOpe Media System

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

2977754-000-02

SARTORIUS

Contents

1 Introduction

4Cell® SmartCHOpe Media System has been designed as perfusion medium and consists of four different media:

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

These cell culture media are chemically defined non-animal origin growth media formulated to maximize the product titer in CHO DG44 Cell lines and cell lines of other CHO lines for perfusion. The perfusion medium needs to be prepared by combining Production Medium (PM) with both feeds and provides as liquid perfusion medium robust performance in production systems of small and large scale, e. g. Ambr® 250 and high-throughput perfusion 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.

1.1 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® SmartCHOpe Stock and Adaptation Medium (SAM) and 4Cell® SmartCHOpe 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® SmartCHOpe Feed Medium A (FMA) and 4Cell® SmartCHOpe Feed Medium B (FMB) 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® SmartCHOpe Media System components at 2 – 8°C protected from light.

1.4 Recommended Materials

- 125 mL Erlenmeyer flask, Ambr® 15 | 250, 2L – 50L bioreactors
- 100 – 400 g/L sterile filtered Glucose
- L-Glutamine, 200 mM
- $NaHCO_3$
- Pluronic-F68
- PES membrane filter with 0.1 µm pore size, e. g. Sartopore® 2

For the filtration of cell culture medium following filter sizes are recommended:

Volume [L]	Filter	Size	Order-No.
0 – 5	Sartopore® 2 Midicaps® XLM	7	5445358M7--OO--A
6 – 10	Sartopore® 2 Midicaps® XLM	8	5445358M8--OO--A
11 – 50	Sartopore® 2 Midicaps® XLM	9	5445358M9--OO--A
51 – 100	Sartopore® 2 Midicaps® XLM	0	5445358M0--OO--V
101 – 200	Sartopore® 2 T-Style Maxicaps® XLM	1	5448358M1G-OO
201 – 500	Sartopore® 2 T-Style Maxicaps® XLM	2	5448358M2G-OO
501 – 1000	Sartopore® 2 T-Style Maxicaps® XLM	3	5448358M3G-OO

2 Instructions for Use

2.1 Adapting Cell Lines to 4Cell® SmartCHOpe 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® SmartCHOpe Stock and Adaptation Medium (SAM). Choose a high seeding cell density at each passage (e. g. 3 x 10^5 cells/mL) for a minimum of two weeks. When the cells achieve a stable growth rate and viability >90% for two passages, the adaptation is considered complete.
- **Option 2:** Passage the culture into a mixture of original culture medium and 4Cell® SmartCHOpe SAM and gradually increase the content of 4Cell® SmartCHOpe SAM. An example for a stepwise adaptation protocol is given below.

Adaptation step	Ratio of original medium to 4Cell® SmartCHOpe 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® SmartCHOpe 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 1 – 2 days and dilute the culture to a suitable seeding density with fresh pre-warmed medium (e. g. 2 – 3 x 10^5 viable cells/mL).

2.3 Thawing of Cells | Initiation of Culture Process

1. Pre-warm 4Cell® SmartCHOpe Stock and 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® SmartCHOpe 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® SmartCHOpe 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 = 10^3 x (50/orbital diameter)$^{1|3}$

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® SmartCHOpe Stock and Adaptation Medium (SAM) with 7.5% Dimethylsulfoxide (DMSO). L-Glutamine can be added optionally (4 – 6 mM final concentration) to the freezing medium. 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 gently 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.
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. Store 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 Perfusion Mode

The 4Cell® SmartCHOpe Media System includes all necessary medium components for adaptation of CHO cell lines, cultivation from stock culture of adapted cells up to 50L of perfusion cultivation in 4Cell® SmartCHOpe Production Medium (PM) plus Feed Medium A (FMA) and Feed Medium B (FMB). Stirred bioreactors and Sartorius Ambr® 250 are ideally suited to carry out comprehensive evaluation of perfusion parameter in the course of process development.

The Table 1 below outlines volumes needed for each individual medium component per 10L of ready-to-use perfusion medium, prepared according to the recommended protocols in chapter 3, page 12.

NOTE

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

Component	10L
PM	9.12L
FMA	0.8L
FMB	0.08L

Table 1: Component 10L.

2.6 Perfusion Protocol

The following protocol is best carried out in a stirred tank bioreactor; alternatively, Ambr® 250 high-throughput perfusion system can be used. Semi-perfusion with one manual volume exchange per day may be carried out using Ambr® 15 system or shake flasks.

After successful adaption, prepare the perfusion bioreactor for inoculation. If an external cell retention device is used, it should be connected to the bioreactor and flushed with pre-warmed medium prior to inoculation.

After preparation of the perfusion bioreactor, seed the cells in the desired volume of perfusion medium at a suitable viable cell density (recommended: 2×10^5 cells/mL). If the volume of inoculum is less than 20% of the final volume, cells can be transferred directly from seed medium to perfusion medium; otherwise it is recommended to centrifuge the cells to remove the used seed medium and reconstitute them in fresh perfusion medium.

From day 0 to day 3 a cultivation in batch mode is recommended. If an external cell retention device is used, the external perfusion loop should be started on day 3 without removing permeate through the cell retention device.

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

Begin the perfusion on day 3 after inoculation, or when the viable cell density reaches 2.5 x 10^6 cells/mL. Alternatively, inoculation of the bioreactor at 2.5 x 10^6 cells/mL and immediate start of perfusion is possible.

A suggested perfusion and feeding profile is outlined in the Table 2 below. Other cultivation conditions are the same as outlined above in paragraph "Cell cultivation".

If a process duration of more than 7 days and | or a constant cell density is desired, bleeding of the cell culture is required. Therefore, removing of cell broth from the bioreactor and refilling with fresh perfusion medium can be conducted daily or continuously. In both cases the bleeding amount should be considered for the perfusion rate.

Depending on the cell line and desired cell density, addition of base might be necessary to maintain a constant pH during perfusion cultivation. If base addition is needed, the usage of 1 M sodium bicarbonate is recommended.

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%.

Day	0	1	2	3	4	5	6+
PR [1/d]	–	–	–	1	1	1	1 – 3
Glucose	–	–	–	–	–	–	To target concentration, 1 – 4 g/L

Table 2: Starting perfusion rates and glucose addition. The perfusion rate should be adapted based on actual cell density to maintain a suitable CSPR.

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 Reconstitution of 4Cell® SmartCHOpe Media Powder and Preparation of Perfusion Medium

3.1 4Cell® SmartCHOpe Stock and 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® SmartCHOpe Stock and Adaptation Medium and 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 30 min at room temperature.
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. The osmolality value of the liquefied 4Cell® SmartCHOpe Stock and 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® SmartCHOpe 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® SmartCHOpe 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.

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® SmartCHOpe Production Medium is expected to stand within 280 – 340 mOsmol/kg H_2O.

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.

3.3 4Cell® SmartCHOpe 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 on, the volume should be 85% of the final volume.
2. Add 168.78 g/L of the media powder 4Cell® SmartCHOpe 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® SmartCHOpe 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® SmartCHOpe 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® SmartCHOpe 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 10.40 – 10.60 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® SmartCHOpe 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 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.

3.5 Preparation of Ready-to-use 4Cell® SmartCHOpe Perfusion Medium (Liquid)

To prepare ready-to-use 4Cell® SmartCHOpe Perfusion medium, liquefied Production Medium, FMA and FMB need to be mixed at the following ratio: PM : FMA : FMB = 0.912 : 0,08 : 0.008.

If the mixture isn't prepared under sterile conditions, sterile filter the completed medium using a PES membrane filter with 0.2 µm pore size (Sartopore® 2).

4Cell® SmartCHOpe media do **not** contain L-Glutamine. Add L-Glutamine at a final concentration of 2 mM to 6 mM (as required for your process) by supplementing a stock solution (e.g. 200 mM) to the mixture.

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.

Last updated:
12 | 2022

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LM | Publication No.: SCM6017-e221204