Instructions for Use

4Cell® SmartCHO Media System

A Chemically Defined and Animal Component Free Cell Culture Media for any CHO Cell Lines in Batch, Fed-Batch and Perfusion processes

SARTORIUS

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1 Product Description

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

- 4Cell® SmartCHO Stock and Adaptation Medium (SAM)
- 4Cell® SmartCHO Production Medium (PM)
- 4Cell® SmartCHO Feed Medium A (FMA)
- 4Cell® SmartCHO 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 also suitable to other CHO lines for batch, fed batch and perfusion processes. The media system provides robust performance in production systems of small and large scale, e .g. flask, Ambr® 15 | 250 and bioreactors.

2 Features

- Chemically defined (CD) media
- Animal-Component Free (ACF) media
- Serum-free (SF) media
- Protein-free (PF) media
- Does not require the addition of serum.
- Does not contain antibiotics.
- The 4Cell® SmartCHO SAM and PM media require the addition of Glutamine.
- Available as non-sterile powder and as sterile liquid.

3 Intended Use and Safety

For the intended use, please refer to the 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.

4 Storage and Stability

4.1 Stability

4Cell® SmartCHO Stock and 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.
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.

4.2 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 – 8°C protected from light.

4.3 Recommended Materials

- DMSO for cell preservation
- 125 mL Erlenmeyer flask, Ambr® 15 | 250, 2L – 50L bioreactors
- 100 – 400 g/L sterile filtered Glucose
- L-Glutamine, 200 mM
- NaHCO3
- Pluronic-F68
- PES membrane filter with 0.1 µm or 0.2 pore size, e.g. Sartopore® 2 (5441307H5--OO--B)

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

Table 1: Recommended filters sizes

5 Instructions for Use

5.1 Adapting Cell Lines to 4Cell® SmartCHO Medium

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

- Option 1: Directly transfer the culture from the initial medium into 4Cell® SmartCHO Stock and 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 two 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 stepwise 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

5.2 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 - 3 days) and dilute the culture to a suitable seeding density with fresh pre-warmed medium (e.g 3-4 x 10^5 viable cells/mL).

* 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)

5.3 Thawing of Cells | Initiation of Culture Process

1. Pre-warm 4Cell® SmartCHO 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® 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 an inoculum into the culture vessel. Proceed with cell cultivation as described above.

5.4 Freezing 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 and Adaptation Medium (SAM) with 7.5% Dimethyl sulfoxide (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 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.

5.5 Protein Production in Fed-Batch Mode

The 4Cell® SmartCHO Media System 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 a comprehensive evaluation of fed-batch parameters during process development.

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

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

Table 2: Volumes needed for each individual medium component dependent on the target cultivation volume from seed train to the end of fed-batch culture

If it is not possible to measure the level of glucose daily, contact your sales representative for more information about the feed optimization tool, whereby feeds are added and monitored depending on the glucose target.

5.6 Fed-Batch Protocol

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

1. 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 x 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.
2. From day 3 after inoculation, perform daily sampling and monitoring cell density, viability, product titer, and key metabolites.
3. 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 x 10^6 cells/mL. Suggested feeding options for a fed-batch are outlined in Tables 3a – b below. Other cultivation conditions are the same as outlined above in paragraph “Cell cultivation”.

4. 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. 4Cell® SmartCHO FMB has a pH of above 10, so its addition may lead to a short spike in culture pH.
5. 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.
6. 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%.

Tables 3 to 5: Three suggested options for a fed-batch procedure to determine the optimal daily feeding rate. The ratio of 4Cell® SmartCHO FMA to 4Cell® SmartCHO 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**	**10**	**11**	**12**	**13**
FMA	–	–	–	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%
FMB	–	–	–	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Glucose	-	-	-	-	-	To target concentration, 4 – 6 g/L								

Table 3: 2% feeding strategy

Day	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
FMA	–	–	–	3%	3%	3%	3%	3%	3%	3%	3%	3%	3%	3%
FMB	–	–	–	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Glucose	-	-	-	-	-	To target concentration, 4 – 6 g/L								

Table 4: 3% feeding strategy

Day	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
FMA	–	–	–	4%	4%	4%	4%	4%	4%	4%	4%	4%	4%	4%
FMB	–	–	–	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%	0.4%
Glucose	-	-	-	-	-	To target concentration, 4 – 6 g/L								

Table 5: 4% feeding strategy

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

5.7 Protein Production in Perfusion Mode

The 4Cell® SmartCHO Media and Feeds has been designed to support a range of continuous or steady-state perfusion culture applications, including small-scale perfusion-mimic systems, N-1 High-Inoculation Fed-Batch and perfusion-capable bioreactors. The medium is designed to support cell growth and productivity at 1-2 vessel volume per day (VVD).

Table 6 below outlines volumes needed for each individual medium component per 10 L of ready-to-use perfusion medium, prepared according to the recommended protocols in chapter 3.

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

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

Table 6: Component 10L.

5.8 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.

1. After successful adaptation, 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.
2. After preparation of the perfusion bioreactor, seed the cells in the desired volume of 4Cell® SmartCHO Production Medium (PM) at a suitable viable cell density (recommended: 0.3 x 10^6 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.
3. From day 0 to day 3, 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.
4. From day 3 after inoculation, perform daily sampling and monitoring cell density, viability, product titer, and key metabolites.
5. Begin the perfusion on day 3 after inoculation, or when the viable cell density reaches approx. 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 are outlined in Table 7 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 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.
6. 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+
VVD [1/d]	–	–	–	1	1	1	1 – 3
Glucose	–	–	–	–	–	–	To target concentration, 1 – 4 g/L

Table 7: Starting perfusion rates (Volume per Volume per Day (VVD)) and glucose addition. The perfusion rate should be adapted based on actual cell density to maintain a suitable CSPR.

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

5.9 N-1 High-Inoculation Fed-Batch Protocol

N-1 stage in rocking motion system

For inoculation of a 5 L high-inoculation fed-batch process, a high number of cells are needed. For this, it is very profitable to choose a process and system, in which you get the needed cell number fast and in the best controlled state to make sure the cells are most viable for the n-stage process.

For n-1 stage, cells are cultivated in a 1 L working volume perfusion bag using the Rocking Motion RM20/50 system with defined process parameter setpoints like

- pH (7.00), DO (40 %), temperature (36,8 °C), rocking angle (10 °), rocking rate (20-42 rpm, dynamic).

Media Exchange rate needs to be tested | optimized for the specific cell line

- e.g. cell-specific perfusion rate (50 pL/cell/day) or VVD ranging from VVD1 to VVD2.5.

Processes may be controlled by MFCS software to ensure a continuous media exchange throughout the cultivation time. Alternatively, pump rates for harvesting and feeding may also be set manually.

1. The perfusion bag is inoculated with a seeding density of 0.6 x 10^6 cells/mL in 4Cell® SmartCHO Production Medium (PM).
2. Start perfusion using SmartCHO perfusion media when the viable cell density reaches 2.5 x 10^6 cells/mL.
3. Each day, we recommend taking a sample for offline analysis of cell growth parameters and metabolites e.g. in Vi-CELL XR Cell Viability Analyzer and BioProfile FLEX2 devices respectively (similar devices are possible).
4. Glucose level is controlled by adding an additional amount into the perfusion media, e.g. 5 g/L.
5. Cells are expanded to a certain cell number which enables the operator to inoculate the n-stage - in this case a 5 L Univessel® - with a cell concentration of 10 x 10^6 cells/mL. For transferring the cells, use a sterile bottle and connect it to the perfusion bag. Drain sufficient volume of cell broth from the bag into the bottle, adjust the volume you will need for n-stage inoculation and inoculate the Univessel® by connecting the bottle to the bioreactor.

N-stage: high-inoculation fed-batch in 5 L Univessel®

Starting volume of the n-stage process is 3 L of the same media as in the n-1 process

- 4Cell® SmartCHO: 91.2 % PM, 8 % FMA and 0.8 % FMB

Temperature, DO and pH set points are equal to those used in the rocking motion system

- pH 7.00, DO 40 %, temperature 36,8 °C

A down-flow stirring speed of 270 rpm is applied using two 3-blade segment impellers.

1. From day 1 onwards, the culture is fed with 5.2 % FMA and 0.52 % FMB of culture start volume (1.3x) daily.
2. Feed glucose bolus as needed on demand also from day 1 on, using a 400 g/L glucose stock solution.

3. On days 3 - 5, a temperature shift can be performed (optional, cell line dependent).
4. Each day, we recommend taking a sample for offline analysis of cell growth metabolites, e.g. in Vi-CELL XR Cell Viability Analyzer and BioProfile FLEX2 device respectively (similar devices are possible).
5. The process ends either on day 12 or as soon as viability drops below 70 %.
6. Feeding strategy and temperature shift might need to be optimized for the respective cell line (refer to the feed optimization tool for more support).

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

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

6.1 4Cell® SmartCHO 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 powder 4Cell® SmartCHO Stock and 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 30 min at room temperature, or until all powder is dissolved.
4. Add 1.80 g/L NaHCO3 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 and should therefore be avoided. The osmolality value of the liquefied 4Cell® SmartCHO Stock and Adaptation Medium is expected to stand at 270 – 330 mOsmol/kg H2O.
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.

6.2 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 NaHCO3 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 NaHCO3 will lead to a gradual increase in pH and should therefore be avoided. The osmolality value of the liquefied 4Cell® SmartCHO Production Medium is expected to stand within 280 – 340 mOsmol/kg H2O.
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.

6.3 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 H2O, 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.

6.4 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 at 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® SmartCHO Feed Medium B is expected to stand within 185 – 225 mOsmol/kg H2O, 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.

6.5 Preparation of Ready-to-use 4Cell® SmartCHO Perfusion Medium (Liquid)

To prepare ready-to-use 4Cell® SmartCHO 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® SmartCHO 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.

7 Contacts

Contact your Sales representative for further information.

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