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Technical Datasheet

CHOin1™

Serum Free CHO Medium,
Animal component free, chemically defined
With Pluronic F-68®
Without L-Glutamine and Sodium bicarbonate

Product Code: SFM007AP

Product description:

Serum free media are designed to grow a specific cell type or perform a specific application in absence of serum. Unlike serum supplemented media which may be utilized for a broad range of cell types and culture conditions, serum free media are specific.

SFM007AP is a animal component-free and chemically defined serum-free CHO medium devoid L-glutamine, sodium bicarbonate and phenol red. The medium is formulated with 0.1% Pluronic® F-68 to protect against mechanical shear damage.

Contents:

Code	Contents
Part A	Basal Medium, powder
Part B	Growth Supplement

Directions:

Preparation of basal medium:

1. Suspend 21.9 gms of Part A in 900ml tissue culture grade water with constant stirring until powder is completely dissolved. Do not heat the water.
2. Add 1.6gms of sodium bicarbonate powder (TC230) or 21.3ml of 7.5% sodium bicarbonate solution (TCL013) for 1 litre of medium and stir until dissolved. *For GS clone: Do not supplement it with L- Glutamine For CHO K_1 & DHFR Negative clones: Supplement L-Glutamine at 4 - 8mM*
3. Adjust the pH to 0.2 - 0.3 units below the desired pH using 1N HCl or 1N NaOH since pH tends to rise during filtration.
4. Make up the volume to 1000ml with tissue culture grade water.
5. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using a positive pressure rather than vacuum to minimize the loss of carbon dioxide.

Preparation of complete medium (SFM007AP):

1. Thaw the growth supplement (Part B) overnight at 2-8°C.
2. Disinfect the external surface of bottle of Part A and Part B by spraying with isopropyl alcohol before placing in a biosafety hood.
3. Transfer the entire content of one bottle of Part B to 1 litre of basal medium (Part A) under aseptic conditions and swirl gently to mix.
   Note: If desired, 10ml of Antibiotic-Antimycotic solution (A002) can be added to 1litre of complete medium.

   Note:

   For Glutamine Synthetase Expression System, do not add L-glutamine. Supplementation with GS supplement is not required.
4. Store the complete medium (SFM007AP at 2-8°C until use.

Procedure for Adaptation:

CHO cells can be adapted to SFM007AP by direct adaptation from original serum- free media or gradual weaning.

Critical points:

- Cells used for adaptation should exhibit a healthy morphology and have more than 90% viability.
- Cells should be in the mid-logarithmic phase of growth.
- It is necessary subculture the cells at least thrice at each step, before going to the next step of adaptation.
- Subculturing should be performed when the cells are 70 – 80% confluent.
- This procedure is applicable for adaptation of CHO cells from existing serum free medium to CHOin1™ medium

Direct Adaptation:

1. Subculture the cells from original free serum medium directly into SFM007AP with a seeding density of 0.5 X 10^6 cells/ml.
2. Incubate at 37°C in a humidified atmosphere with 5-10% CO_2 until viable count reaches 1 X 10^6 cells/ml. It might be required to give medium change with fresh SFM007AP after 2 days.
3. Subculture in fresh SFM007AP with normal seeding densities.
4. Maintain cells in SFM007AP for several passages till the cells are completely adapted to SFM007AP.

Gradual weaning procedure

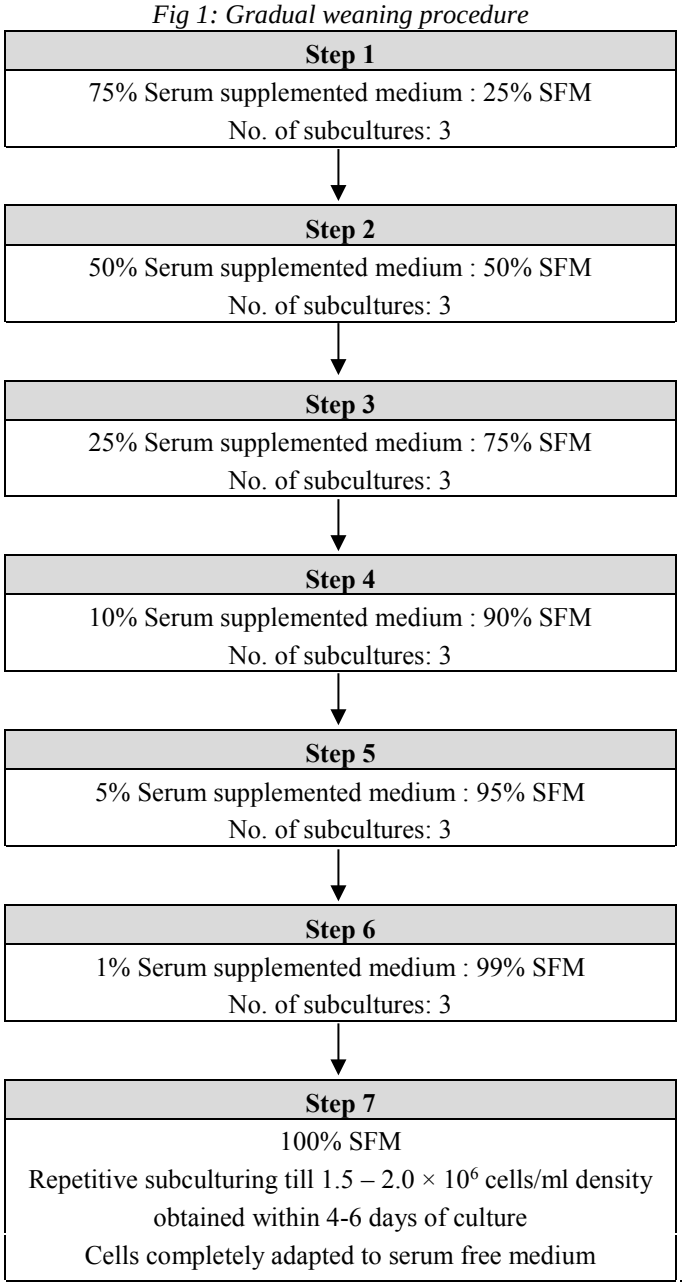
For Static culture:

1. Subculture the cells from original free serum medium and seed them in 75:25 ratio of original free serum medium and SFM007AP with a seeding density of 0.3 – 0.5 × 10^6 cells/ ml.
2. Incubate at 37°C in a humidified atmosphere with 5-10% CO_2. Make provision for gas exchange by loosening the caps of flasks in case of closed caps or use vented caps.
3. Subculture once the cells become 70-80% confluent.
4. Determine cell density and reseed the cells in 75:25 ratio of serum containing medium and SFM007AP.
   Note: It is necessary to subculture the cells at least thrice at each step of adaptation beore going to the next step.
5. Repeat steps 1 to 4 for 3 subcultures of each step of gradual adaptation.
   Note: Refer figure 1 for details of each adaptation step.
6. After step 3 (25:75 serum containing medium: SFM007AP) of adaptation, the cells cannot be directly subcultured in 100%serum free conditions. Complete withdrawal of serum may alter cell morphology and decrease the cells viability. Hence, it is very critical to maintain them at 10:90, 5:95 and 1: 99 ratios before 100% SFM007AP
7. When the cells reach 100% serum free step of adaptation, subculture them repetitively till a cell density of 1.5 × 10^6 cells/ml is obtained within 4 - 6 days of culture. At this point, the cells are considered to be adapted to SFM007AP.

For Shaker culture:

1. Subculture cells from 5 - 10% serum containing medium directly in to SFM007AP at a 50:50 ratio with a seeding density of 0.3 to 0.5 x 10^6 cells/ml.
2. Incubate at 37°C in a humidified atmosphere with 5 - 10% CO_2 and agitate at desired speed (in rpm) on an orbital shaker platform.
3. Subculture once cell density exceeds 1 x 10^6 cells/ml. Always centrifuge cells during adaptation process. It might be required to subculture cells multiple times at each step.
4. Resuspend the cells by gradually increasing the ratio of SFM007AP to the original medium (75:25). Repeat step 2 and 3.
5. Resuspend the cells at 90:10 ratio of SFM007AP to original medium. Repeat step 2 and 3.
6. Finally resuspend the cells in 100% SFM007AP. Repeat till you get a uniform cell distribution without any clumps at a density of 1.5 to 2 x 10^6 cells/ml within 4 - 6 days of culture. At this point, you can consider the cells to be adapted to serum free conditions in SFM007AP.

Fig 1: Gradual weaning procedure



Material required but not provided:

L-Glutamine 200mM Solution (TCL012)
HT Medium Supplement 50X liquid (TCL073)
Trypsin – EDTA solution 1X (TCL007)
Trypsin Inhibitor from Soyabean 1X (TCL068)

Quality control:

Appearance
Part A: White to off-white homogenous powder

Part B: Colorless clear solution

Solubility
Clear light pink colored solution at 21.9 gms/L

pH of Part A without sodium bicarbonate
6.60 to 7.20

pH of part A with sodium bicarbonate
7.00 to 7.60

Osmolality of Part A without sodium bicarbonate (mOsm/KgH_2O)
250.00 – 290.00

Osmolality of Part A with sodium bicarbonate (mOsm/KgH_2O)
285.00 – 325.00

Cultural Response
The growth promotion capacity of the medium is assessed quantitatively by estimating the cell counts.

Endotoxin content
Less than 1EU/ml

Storage and shelf life:

Store basal medium at 2-8ºC away from bright light.
Store serum free growth supplement at -20ºC.
Shelf life of Part A is 36 Months and Part B is 12 Months after reconstitution the shelf life of complete medium is 8 weeks at 2-8ºC.

Use before expiry date given on the product label. **Note**: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

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