CHOin1™

Chemically defined,
Animal Component Free,
Serum free CHO Medium and Feed

HIMEDIA®
For Life is Precious

CELLBIO™

Product Portfolio

Expansion	
CHOin1™ Serum Free CHO Medium, Chemically defined, Animal Component Free, w/ pluronic F-68® w/o L-Glutamine and Sodium bicarbonate	SFM007AP-1L SFM007AP-10L
CHOin1™ Serum Free CHO Medium, Chemically defined, Animal Component Free, w/ pluronic F-68® and Sodium bicarbonate w/o L-Glutamine	SFM007L-500ML SFM007L-1000ML
CHOin1™ Feed Medium Chemically defined, Animal Component Free, w/ D-Glucose and pluronic F-68® w/o L-Glutamine and Phenol red	SFF006P - 1L SFF006P - 5L
CHOin1™ Feed Medium, Liquid Chemically defined, Animal Component Free, w/ D-Glucose and pluronic F-68® w/o L-Glutamine and Phenol red	SFF006L - 1L

CHOin1™

Chinese hamster ovary (CHO) cells are the preferred expression system for recombinant therapeutic protein production. One of the characteristics of CHO cells is their ability to adapt to serum-free medium and to be cultured in chemically defined medium devoid of animal-derived components.

Furthermore, CHO cells are superior to other hosts like Escherichia coli and Saccharomyces cerevisiae in that they can fold and assemble proteins correctly and carry out post-translational modifications like glycosylation. Particularly, recombinant therapeutic proteins and monoclonal antibodies (mAbs) as adalimumab, bezlotoxumab, avelumab, etc. are produced using CHO cells.

CHO cells detach from the surface and float when cultured in serum-free medium, allowing for large-scale high-density cell cultures in bioreactors. In the absence of serum, serum-free media are designed to grow a specific cell type or perform a specific application. Serum free media, as opposed to serum supplemented media, can be used for a wide variety of cell types and culture conditions.

CHOin1™ serum-free media & feed are designed to support the proliferation, productivity of CHO clones. The media and feeds are suitable for research and routine large-scale GMP manufacturing and are ideal for protein production using CHO-DG44, CHO-K1, CHO-S, and CHO-GS cell lines.

Regulatory concerns minimized

Chemically defined media do not contain any components of human and animal origin, which brings batch uniformity and ease of upstream and downstream processing.

Being chemically defined, these media also eliminate the risk of contamination by animal pathogen thereby making the manufacturing process safer.

Scalability

Carefully designed for growth of specific cell types, these serum free media are suitable for culture systems at laboratory scale applications and in large bioreactor scale production runs.

CHOin1™ (SFM007AP)

Product Specifications

Part A: White to off-white homogeneous powder

Part B: Frozen Supplement

Intended use

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.

Directions for Use

Ingredient	SFM007AP
Part A	23 g in 900ml cell culture grade water
Sodium bicarbonate	1.6g sodium bicarbonate powder or 21.3ml of 7.5% sodium bicarbonate solution
pH adjustment	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.
Volume make up	Make up the volume to 1000ml with water.
Filter sterilization	Filter sterilize the medium immediately by filtering through a sterile membrane filter with porosity of 0.22 micron or less, using a positive pressure.
Complete Media Preparation	Thaw the growth supplement (Part B) overnight at 2-8°C. 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. *Aseptically add sterile supplement as per the clone being used.
Antibiotic - Antimycotic solution	If required, 10ml of sterile antibiotic-antimycotic solution (A002) can be aseptically added to 1L of complete medium.
Storage	Store complete medium at 2 – 8°C until use

Quality control

Test	SFM007AP
Appearance	Part A: White to off-white homogeneous powder Part B: Clear to slightly hazy solution
Solubility of part A	Clear light pink colored solution at 23 gms/L
pH of Part A without Sodium bicarbonate	6.70-7.30
pH of Part A with Sodium bicarbonate	7.10-7.70
Osmolality of Part A without Sodium bicarbonate	260-300 mOsm/KgH_2O
Osmolality of Part A with Sodium bicarbonate	290-330 mOsm/KgH_2O
Cultural Response	Passes *
Endotoxin content	Less than 1 EU/ml

***** *Each lot is tested for its ability to expand recombinant CHO cells. Cells from a qualified cryopreserved bank are revived and subcultured for a min of two passages. The cells are then seeded in shake flasks containing test and control media. The culture is continued for 8-10days. Cell density and viability are determined each day and considered to be acceptable according to internal specifications.*

Storage and shelf life

Store basal medium (Part A) at 2-8°C away from bright light.

Store serum free growth supplement (Part B) at -20°C.

Use before expiry date given on the product label. Shelf life of the reconstituted complete medium is 8 weeks at 2-8°C.

Note: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

CHOin1™ (SFM007L)

Product Specifications

Part A: Basal Medium, Liquid

Part B: Growth Supplement

Intended use

SFM007L is a animal component-free and chemically defined serum-free CHO medium which contains Pluronic® F-68 and sodium bicarbonate. It does not contain L-glutamine. The medium is formulated with 0.1% Pluronic® F-68 to protect against mechanical shear damage.

Directions for Use

Ingredient	SFM007L
Complete Media Preparation	Thaw the growth supplement (Part B) overnight at 2-8°C. Transfer the entire content of one bottle of Part B basal medium (Part A) under aseptic conditions and swirl gently to mix. *Aseptically add sterile supplement as per the clone being used.
Antibiotic - Antimycotic solution	If required, 10ml of sterile antibiotic-antimycotic solution (A002) can be aseptically added to 1L of complete medium.
Storage	Store complete medium at 2 – 8°C until use

Quality control

Test	SFM007L
Appearance	Part A: Clear light pink colored solution Part B: Clear to slightly hazy solution
pH of Part A	7.00-7.60
Osmolality of Part A	280.00-320.00 mOsm/KgH_2O
Sterility	No bacterial as fungal growth is observed after 14 days of incubation as per USP specification.
Cultural Response	The growth promotion on capacity of the medium is assessed quantitatively by estimating the cell counts.

* *Each lot is tested for its ability to expand recombinant CHO cells. Cells from a qualified cryopreserved bank are revived and subcultured for a min of two passages. The cells are then seeded in shake flasks containing test and control media. The culture is continued for 8-10days. Cell density and viability are determined each day and considered to be acceptable according to internal specifications.*

Storage and shelf life

Store basal medium (Part A) at 2-8°C away from bright light.

Store serum free growth supplement (Part B) at -20°C.

Use before expiry date given on the product label. Shelf life of the reconstituted complete medium is 8 weeks at 2-8°C.

Note: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

CHOin1™ Feed Medium (SFF006P)

Product Specifications

Part A: White to off-white homogeneous powder

Part B: Frozen supplement

Intended use

CHOin1™ Feed Medium is a chemically defined, animal component free powder feed supplement designed to enhance cell growth and monoclonal antibody production of recombinant Chinese Hamster Ovary (CHO) cells in fed batch mode of protein production.

Directions for Use

Ingredient	SFF006P
Part A	97.1g in 700ml cell culture grade water. Stir for 30 -40 minutes on a magnetic stirrer.
pH adjustment	Adjust the pH to 11 using 5N sodium hydroxide with continuous stirring. Powder dissolves completely and solution appears clear. Now adjust it to pH 7.1-7.3with 5N HCl.
Volume make up	Make up the volume to 1000ml with water.
Filter sterilization	Filter sterilize the complete medium immediately by filtering through a sterile membrane filter with porosity of 0.22 micron or less, using a positive pressure.
Complete media preparation	Thaw the growth supplement (Part B) overnight at 2–8°C. Transfer the entire content of one bottle of Part B to 1 litre of Part A under aseptic conditions and swirl gently to mix.
Supplements	Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers as per the clone being used.
Storage	Store complete feed medium at 2–8°C until use

Quality control

Test	SFM007AP
Appearance	Part A: White to off-white homogeneous powder Part B: Colorless clear solution
pH (Adjusted)	7.00-7.60
Cultural Response	Passes *

***** *Each lot is tested for its ability to expand recombinant CHO cells. Cells from a qualified cryopreserved bank are revived and subcultured for a min of two passages. The cells are then seeded in shake flasks containing control media. Feed is added as per decided internal feeding strategy. The culture is continued for 10-14days. Cell density and viability are determined each day and considered to be acceptable according to internal specifications.*

Storage and shelf life

Store Feed medium at 2-8°C away from bright light.

Store Feed supplement at -20°C. 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 2-8°C.

Use before expiry date given on the product label.

CHOin1™ Feed Medium (SFF006L)

Product Specifications

Part A: Feed Medium, Liquid

Part B: Feed supplement

Intended use

CHOin1™ Feed Medium is a chemically defined, animal component free powder feed supplement designed to enhance cell growth and monoclonal antibody production of recombinant Chinese Hamster Ovary (CHO) cells in fed batch mode of protein production.

Directions for Use

Ingredient	SFF006L
Complete media preparation	Thaw the growth supplement (Part B) overnight at 2–8°C. Transfer the entire content of one bottle of Part B to Part A under aseptic conditions and swirl gently to mix.
Supplements	Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers as per the clone being used.
Storage	Store complete feed medium at 2–8°C until use

Quality control

Test	SFM007AP
Appearance	Part A: Amber coloured clear solution Part B: Clear colorless solution
pH (Adjusted)	7.00-7.60
Cultural Response	Passes *

* *Each lot is tested for its ability to expand recombinant CHO cells. Cells from a qualified cryopreserved bank are revived and subcultured for a min of two passages. The cells are then seeded in shake flasks containing control media. Feed is added as per decided internal feeding strategy. The culture is continued for 10-14days. Cell density and viability are determined each day and considered to be acceptable according to internal specifications.*

Storage and shelf life

Store Feed medium at 2-8°C away from bright light.

Store Feed supplement at -20°C. 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 2-8°C.

Use before expiry date given on the product label.

Annexure I: Adaptation Procedure for SFM007AP / SFM007L

Adaptation pre-requisites for initial cell inoculum

To adapt the cells to SFM successfully, it is critical that the culture has -

- Healthy morphology
- More than 90% viability
- Mid-logarithmic phase of growth

Critical Experimental Conditions

- Cells should have more than 90% viability at each passage
- Centrifugation speed should not exceed 800 – 1000rpm
- If viability drops below 90% at any stage of adaptation, give medium change with fresh medium
- If viability does not improve even after changing the medium, discard the culture & start with the fresh cell inoculum

Approach & cultural requirements

	Direct Adaptation	Gradual Adaptation
Definition	Cells switched from serum-supplemented medium into SFM or cells switched from existing serum-free medium to SFM.	Gradual weaning is a slow procedure that involves decreasing the percentage of serum in the medium, thereby gradually adapting the cells to serum free conditions
Initial cell inoculum	~ 15 million cells for one flask	~ 10 million cells for one flask
Shake flasks (125ml)	6 Nos (2 flasks per passage X 3 passages = 6 flasks)	30 Nos (2 flasks per step X 3 passages = 6 flasks per step)
Volume of complete media	200 – 300ml*	400 - 500ml*

* *Media volume may vary depending on the frequency of media changes required*

Direct adaptation

Recommended for adaptation of cells from **existing serum free medium to CHOin1™** Serum Free Medium (SFM007AP) / (SFM007L)

Steps	Requirements	Adaptation from cryopreserved culture	Adaptation from proliferating culture	Critical check-points	Acceptance criteria
1. Cell harvesting from existing serum-free medium	1. Shake flasks (2 Nos) 2. Complete media (2X30ml) 3. 15 million cells (For 1 flask)*	Revive the cells directly in 15ml complete SFM007AP / SFM007L	Harvest the cells from existing serum-free medium in mid-log phase of growth	—	Viability > 90% *If viability is found to be less than 90%, discard the culture and use fresh culture*
2. Centrifugation	1. Centrifuge tubes (2X50ml)	Centrifuge at 800-1000 rpm for 10 minutes		Do not exceed the centrifugation speed & time	—
3. Resuspension	1. Complete media (2X30ml)	Carefully discard the supernatant by gentle aspiration without disturbing the pellet. Resuspend the pellet by pipetting gently with serological pipette, to get a homogenous mixture.		—	—
4.Cell density and viability	1. Trypan blue solution 2. Hemocytometer	Count the cells and make a note of cell count and viability		—	Viability > 90% *If viability is found to be less than 90%, discard the culture and use fresh culture*
5. If viability is less than 90%	1. Complete media (2X30ml)	Give medium change with existing medium to enhance viability		—	If viability does not enhance even after medium change, discard the culture and start with the fresh culture
6. Reseeding	1. Complete media (2X30ml) 2. Shake flasks (2 Nos) 3. 15 million cells (For 1 flask)	Seed with 0.5 × 106/ml density in 125ml shake flask containing 30ml complete CHOin1		—	—
7. Incubation	CO_2 incubator shaker	Incubate the cells at 37°C and 5% - 8% CO_2 at 100-120rpm		Recommended centrifugation speed = 800 – 100rpm. You can optimize if required	—
8. Cell density and viability	1. Trypan blue solution 2. Hemocytometer	Count the cells and make a note of cell count and viability every day		**Viability**	More than 90% *If viability is found to be less than 90%, give medium change*
				Time between two passages	3 – 5 days
				Cell density before passaging	2X – 3X of the seeding density = 1 to 1.5 million cells/ml

Steps	Requirements	Adaptation from cryopreserved culture	Adaptation from proliferating culture	Critical check-points	Acceptance criteria
9. Sub-culturing	1. Centrifuge tubes (2X50ml)	• Centrifuge at 800-1000 rpm for 10 minutes • Carefully discard the supernatant by gentle aspiration without disturbing the pellet. Resuspend the pellet in fresh complete CHOin1 by pipetting gently with serological pipette, to get a homogenous mixture. • Seed in a fresh culture vessel (125ml flask) with 0.5×10^6/ml density		—	—
10. Maintenance	1. Complete media (2X30ml) 2. Shake flasks (2 Nos) 3. 15 million cells (For 1 flask)	Continue maintenance of these cells in the same conditions for total 3 - 5 passages		Viability	More than 90% *If viability is found to be less than 90%, give medium change*
				Time between two passages	3 – 5 days
				Cell density before passaging	2X – 3X of the seeding density = 1 to 1.5 million cells/ml
Adaptation complete Cell Viability more than 90% Cell density 2X to 3X of the seeding density Duration between two subcultures = 3 to 5 days					

Scale up to the higher volume should be performed only after the cells are fully adapted & not mid-way through the protocol

*Direct adaptation from cryopreserved cells – Cell number and revival chart

Cell density per vial (Million cells per vial)	Required cell number per flask	No. of vials to be revived	Acceptance criteria
1	15 million cells	16	Post-revival cell viability should be more than 90% *If it is less than 90%, use different batch of the cryopreserved cells
5		4	
10		2	

Gradual / Sequential adaptation

Recommended for adaptation of cells from

- **Serum-containing medium to CHOin1™** Serum Free Medium (SFM007AP) &
- **Existing serum free medium to CHOin1™** Serum Free Medium (SFM007AP)

Step	% of existing SFM	% of SFM007AP	Seeding density (x 10^6 cells/ml)	No of passages at each stage*	Critical check points before each subculture	Acceptance criteria
1	75	25	0.3	2-3	1. Viability 2. Time between two passages 3. Cell density before passaging	More than 90% 3 – 5 days 2X – 3X of the seeding density = 1 to 1.5 million cells/ml
2	50	50				
3	25	75				
4	10	90				
5	0	100				
Adaptation complete Cell Viability more than 90% Cell density 2X to 3X of the seeding density Duration between two subcultures = 3 to 5 days						

Important: At any stage of gradual adaptation, if viability is found to be less than 90%, and does not enhance on giving medium change, we recommend to start the process from beginning to achieve successful adaptation

HIMEDIA®

For Life is Precious

HiMediaLaboratories™
www.himedialabs.com

CORPORATE OFFICE
Plot No. C-40, Road No. 21Y, Wagle Industrial Area, Thane (West) - 400604, Maharashtra, INDIA.
Tel : +91-22-6147 1919 / 6116 9797 / 6903 4800 | Fax : +91-22-6147 1920
Email : atc@himedialabs.com / info@himedialabs.com