CELLin1™

Chemically defined,
Animal Component Free,
Serum free Virus Production Medium

HIMEDIA®
For Life is Precious

HIMEDIA®
Cell Culture
Enabling Breakthroughs

Product Portfolio

Expansion		
CELLin1™ Chemically defined, Animal Component Free, Serum Free Virus Production medium w/o L-Glutamine and Sodium bicarbonate		SFM036AP-1L SFM036AP-10L
Dissociation		
Trypsin - EDTA Solution 1X 0.25% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline w/o Phenol red For dissociation of Vero, MDCK, MDBK and MRC-5 cells		TCL007-100ML TCL007-500ML
Trypsin Inhibitor from Soyabean 1X, Liquid # w/ 1mg/ml of Trypsin inhibitor in Dulbecco's Phosphate Buffered Saline For Vero, MDCK, MDBK and MRC-5 cells		TCL068-100ML
EnVzyme™ Easy (Animal Free) For dissociation of PK15 cells		TCL137-100ML TCL137-500ML
Cryopreservation		
FREEZin1™ Universal freezing medium w/ DMSO w/o Antibiotics, Antimycotics and Phenol red		TCL098-50ML
Culture Vessels		
	Total culture area (cm^2)	Recommended volume (ml)
HiFactory™, 1 chamber	647 cm^2	200 ml	TCP204-4x1NO TCP204-8x1NO
HiFactory™, 2 chamber	1279 cm^2	400 ml	TCP205-4x1NO TCP205-8x1NO
HiFactory™, 5 chamber	3175 cm^2	1000 ml	TCP206-2x1NO TCP206-4x1NO
HiFactory™, 10 chamber	6335 cm^2	2000 ml	TCP207-2x1NO TCP207-4x1NO
	Surface area (cm^2)	Total Volume (ml)	
Tissue Culture Flask Vented cap	182 cm^2	600 ml	TCG8-4x5NO TCG8-8x5N
Tissue Culture Roller Bottle Close cap	750cm^2	2000ml	TCG9-4x1NO TCG9-12x1NO
Tissue Culture Roller Bottle Vented cap	750cm^2	2000ml	TCG10-4x1NO TCG10-12x1NO
Tissue Culture Roller Bottle Close cap	850cm^2	2000ml	TCG15-4x1NO TCG15-12x1NO
Tissue Culture Roller Bottle, Expanded Surface Close cap	1900cm^2	2000ml	TCG16-4x1NO TCG16-12x1NO
Tissue Culture Roller Bottle Vented cap	850cm^2	2000ml	TCG17-4x1NO TCG17-12x1NO
Tissue Culture Roller Bottle, Expanded Surface Close cap	4350cm^2	5000ml	TCG18-12x1NO

CELLin1™

Chemically Defined, Animal component free, Serum free, Virus production medium
For MDCK, MDBK, PK-15, Vero & MRC-5

In 1995, WHO recommended developing an alternative influenza virus cultivation system. One favoured option is cell culture. In contrast to egg-based production processes, cell-based production technology allows manufacturers to respond to market needs faster and in shorter production cycles and also allows a greater surge capacity, greater process control, and a more reliable and well-characterized product.

For efficient replication of most viruses, it is important that the host cells are actively growing, their doubling time during exponential growth phase is minimum, and death rate is negligible during the growth phase and increase very slowly after this growth phase. Therefore it is very crucial to choose an appropriate culture medium that supports optimum growth of host cells. The use of serum-free synthetic media has increased significantly, particularly when using serum presents a safety hazard and a potential source of unwanted contamination.

Understanding the complexity and key challenges involved in vaccine production process, we have developed a chemically defined, serum-free, animal component free medium - CELLin1™. This medium is suitable for virus cultivation in broad range of cell lines such as Vero, MDCK, MDBK, PK-15 and MRC-5.

Regulatory concerns minimized

Serum free and low protein media do not contain any components of human and animal origin, which brings batch uniformity and ease of upstream and downstream processing.

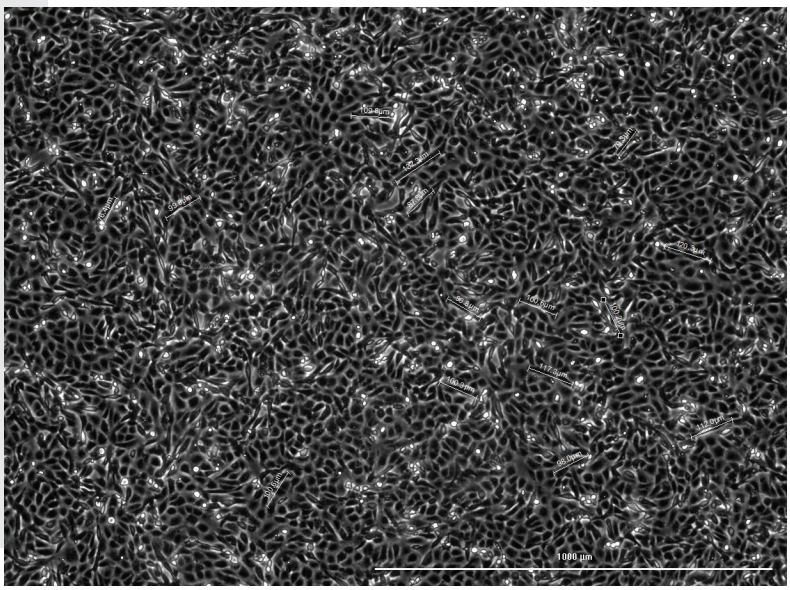
Being chemically defined, these media also eliminate the risk of contamination by animal pathogen thereby making the manufacturing process safer.

Scalability

Carefully designed for growth of specific cell types, these serum free media are suitable for 2D as well as 3D culture systems at laboratory scale applications and in large scale production runs.

Customization

Custom formulations and custom packaging options available to suit your process.



Seed Preparation and Cell Banking

Banking of host cell line and preparation of the seed are the two major preliminary steps involved in viral vaccine production. CELLin1™ supports continuous growth of host cells through multiple subcultures, making it suitable for banking.

Consistent cell growth over serial subcultures

Suitable for cell expansion and banking

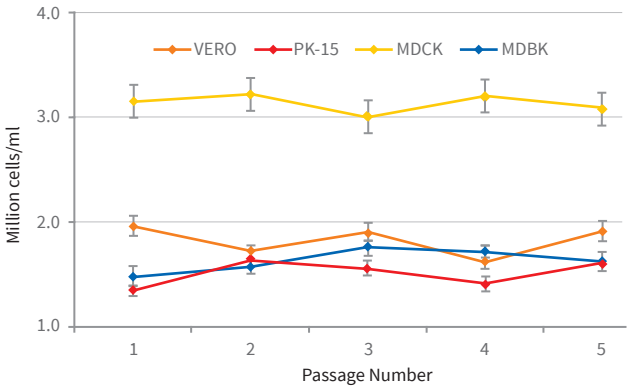


Fig 1 : Vero, PK-15, MDCK and MDBK cells were seeded in CELLin1™ in T175 flask. Consistent cell density was obtained through out 5 subcultures and viability of each cell was more than 90 %, making it suitable for host cell expansion and banking.

Optimum host cell growth

Cultivation period for most viruses in cell line host ranges from 2-4 days. CELLin1™ supports sustained growth of host cells & high cell densities on harvest, thereby assuring optimum virus multiplication.

High viable cell densities

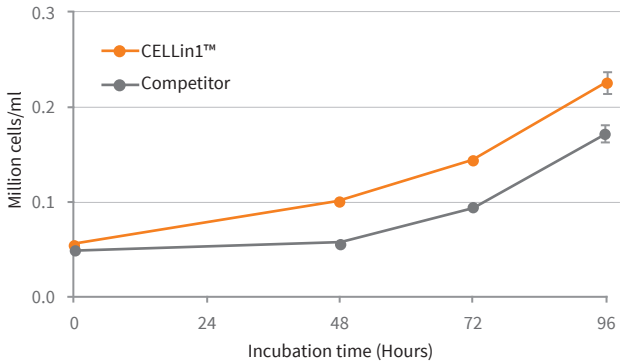


Fig 3 : Vero cells were seeded in CELLin1™ and competitor media at 0.05X10^6 cells/ml in T175. Cells were cultured at 37°C, 5% CO_2, for 4 days. At each time point Viability of cells in CELLin1™ was more than 90 % throughout 4 days.

Flexibility of Production Format

Cells are grown in classical 2D monolayer format during cell banking and expansion phase whereas, 3D microcarrier culturing is performed during actual virus cultivation and vaccine production phase. CELLin1™ has been optimized to support the growth of cells in 2D as well as 3D microcarrier system.

Ability to support 2D and 3D culture expansion

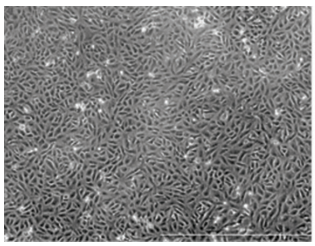
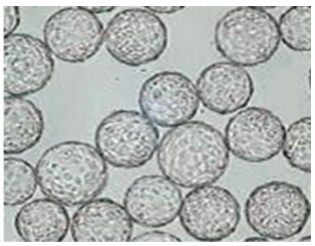


Fig 2 : Vero cells grown in HiFactory™ (TCP204) using CELLin1™ (SFM036AP)
2D Culture



Vero cells grown on Microcarrier beads using CELLin1™ (SFM036AP)
3D Culture

Optimum Virus Productivity

CELLin1™ has been optimized to produce optimum virus titers.

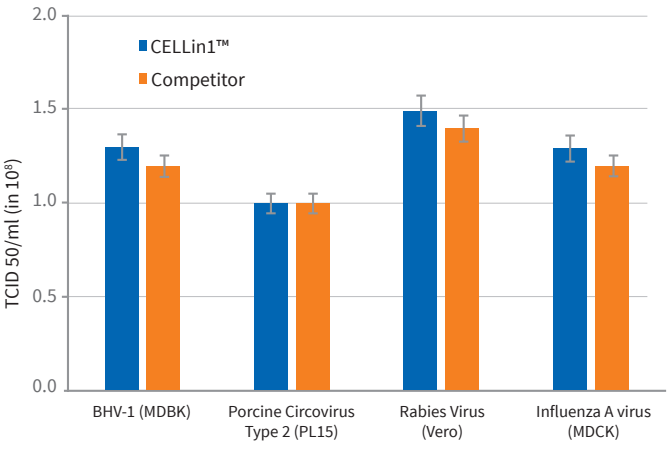


Fig 4 : CELLin1™, exhibited optimum virus production and growth promotion ability in Vero, PK-15, MDCK and MDBK cell lines

Product Specifications

Part A: White to off-white homogeneous powder

Part B: Lyophilized growth supplement

Intended use

CELLin1™ is a chemically defined, animal component-free medium designed for growth and maintenance of Vero, MDCK, MDBK, PK15 and MRC-5 cells.

Directions for Use

Ingredient	**SFM036AP**
Part A	10.8g in 900ml cell culture grade water
Sodium bicarbonate	2.7g sodium bicarbonate powder or 36.0ml of 7.5% sodium bicarbonate solution
L-Glutamine	584mg L-Glutamine powder or 20ml of L-Glutamine 200mM solution
	OR
L-Alanyl-L-glutamine	868.8 mg L-Alanyl-L-glutamine powder or 20ml of L-Alanyl-L-glutamine 200mM solution
Add these ingredients one after the other in 900ml of cell culture grade water with constant stirring	
Part B preparation	Reconstitute lyophilized growth supplement with 10ml cell culture grade water. Mix gently by pipetting up and down. Make sure that the powder is completely dissolved. Add entire content of Part B into 1L of Part A.
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 complete medium immediately by filtering through a sterile membrane filter with porosity of 0.22 micron or less, using a positive pressure.
Antibiotic - Antimycotic solution	If required, 10ml of sterile antibiotic-antimycotic solution (A002) can be aseptically added to 1L of filter sterilized medium.
Storage	Store complete medium at 2 – 8°C until use

Quality control

Appearance

Part A: White to off-white homogeneous powder

Part B: White coloured lyophilized powder

Solubility

Clear solution at 10.8 gms/L

pH of Part A without Sodium bicarbonate

6.10-6.70

pH of Part A with Sodium bicarbonate

7.40-8.00

Osmolality of Part A without Sodium bicarbonate

200-240 mOsm/KgH_2O

Osmolality of Part A with Sodium bicarbonate

260-300 mOsm/KgH_2O

Cultural Response

The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin content

Less than 1 EU/ml

Storage and shelf life

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

Store serum free lyophilized growth supplement (Part B) at 2-8°C.

Use before expiry date given on the product label. Shelf life of the reconstituted complete medium is 4-6 weeks at 2-8°C.

Note: Freezing of the basal medium and complete medium is not recommended.

Procedure for Adaptation

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.

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.

Gradual weaning

1. Subculture the cells from serum containing medium and seed them in 75:25 ratio of serum containing medium and SFM036AP with a seeding density of 0.3-0.5X10^6 cells/ml (STEP 1).
2. Incubate at 37°C in a humidified atmosphere with 5% 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 are 70-80% confluent using EnVzyme™ Easy (TCL137)

   Note: For dissociation of Vero, MDCK, MDBK & MRC-5, use trypsin (TCL007) & inactivate it by adding equal volume of trypsin inhibitor (TCL068). For dissociation of PK-15, use EnVzyme™ Easy (TCL137). It is gentle on cells and does not require neutralization.
4. Determine cell density and reseed the cells in 75:25 ratio of serum containing medium and SFM036AP.

   Note: It is necessary to subculture the cells at least thrice at each step of adaptation before going to the next step.
5. Repeat steps 1 to 4 for three 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: SFM036AP) of adaptation, the cells cannot be directly subcultured in 100% serum free conditions. Complete withdrawal of serum may alter cell morphology and decrease the cell viability. Hence, it is very critical to maintain them at 10:90, 5:95 and 1:99 ratios before 100% serum free conditions.
7. When the cells reach 100% serum free step of adaptation, subculture them respectively till a cell density of 1.5-2.0X10^6 cells/ml is obtained within 4-6 days of culture. At this point, the cells are considered to be adapted to serum free conditions.

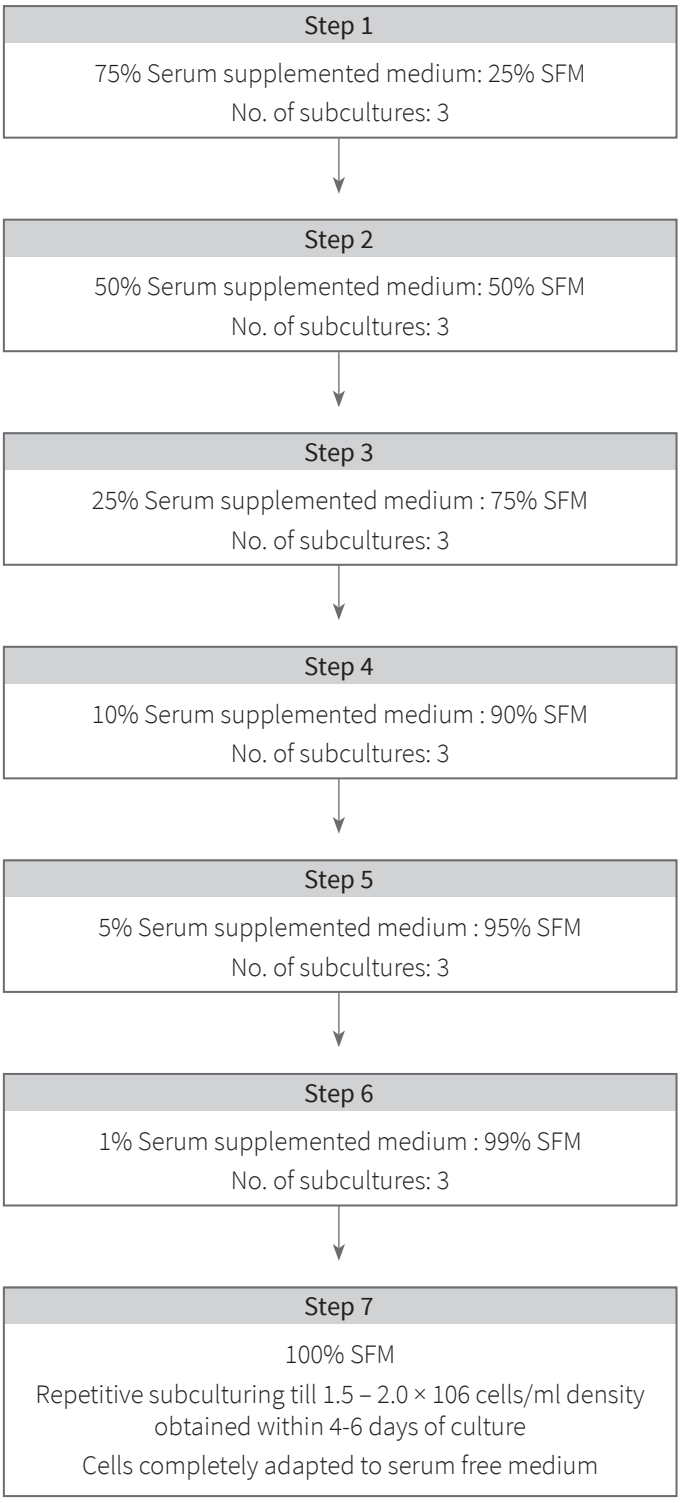


Fig 5: Gradual weaning

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