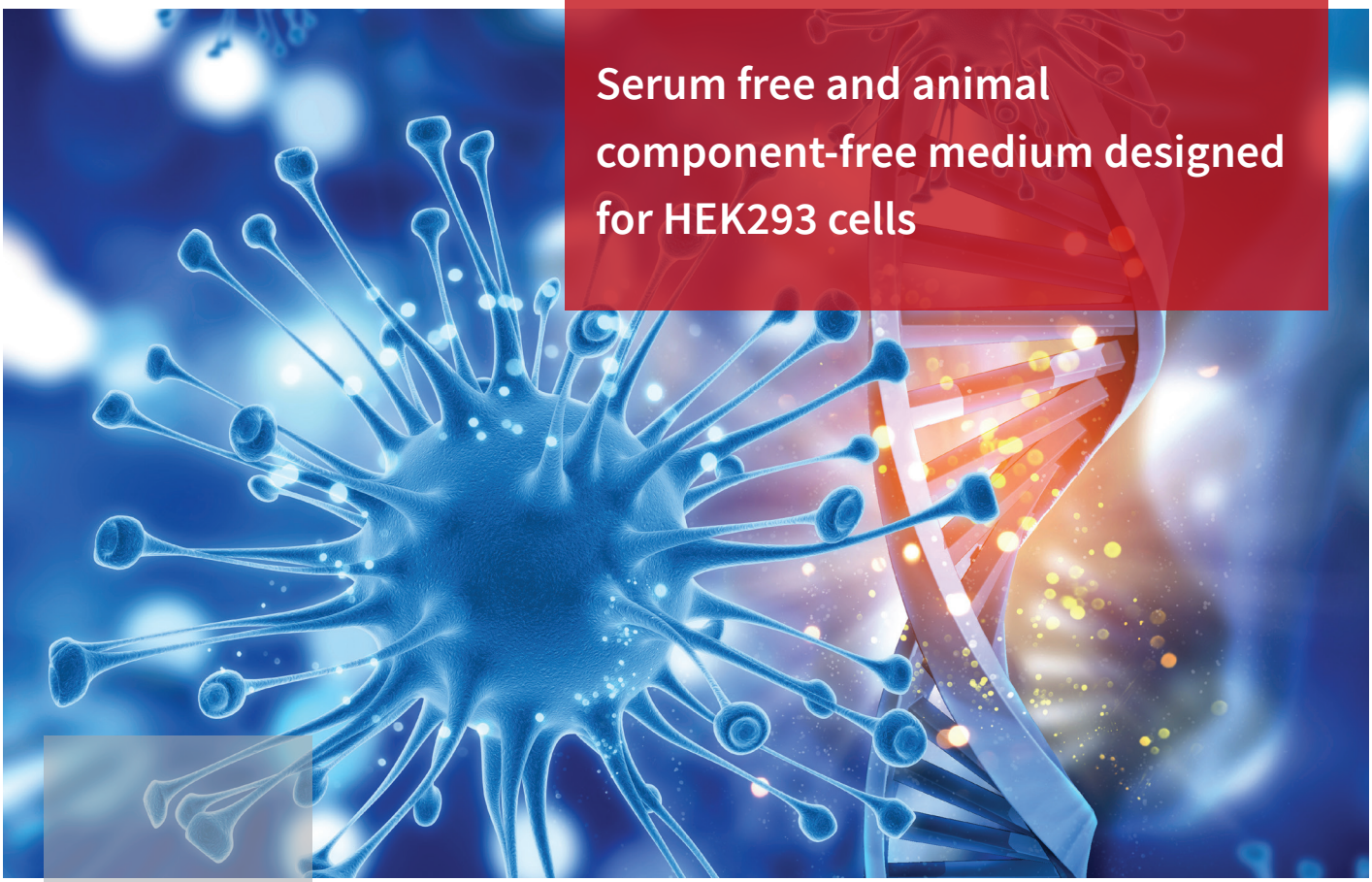
HEKin1™



Serum free and animal component-free medium designed for HEK293 cells

HIMEDIA®
For Life is Precious

HIMEDIA®
Cell Culture
Enabling Breakthroughs

Product Portfolio

Culturing & Expansion

Suspension Culture	
HEKin1™ For suspension culture w/o Sodium bicarbonate, Phenol red and L-Glutamine Contains basal medium in **powder form**	SFM039AP-10L SFM039AP-50L
Adherent Culture	
HEKin1™ For adherent culture w/o Sodium bicarbonate, Phenol red and L-Glutamine Contains basal medium in **powder form**	SFM038AP-10L SFM038AP-50L
L-Glutamine (From non-animal source) Cell Culture Tested	TC243-10G TC243-25G
L-Glutamine 200mM Solution L-Glutamine in 0.85% normal saline Cell culture tested	TCL012-20ML TCL012-100ML
Sodium bicarbonate powder (Sodium hydrogen carbonate) Cell culture tested	TC230-100G TC230-500G
7.5% Sodium bicarbonate solution	TCL013-100ML TCL013-500ML
Dissociation	
Trypsin – EDTA solution 1X 0.25% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline w/o Phenol red	TCL007-100ML TCL007-500ML
Trypsin Inhibitor from Soyabean 1X w/ 1mg/ml of Trypsin inhibitor in Dulbecco's Phosphate Buffered Saline	TCL068-100ML

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

Pluronic F-68® is a registered trademark of BASF

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HEKin1™

HEK293 is one of the most commonly used human cell lines for the production of biotherapeutics proteins and a cell line of choice for vector-based viral vaccine production.

There are many advantages to using HEK293 cells, the major ones being –

- Easy to transfect
- Divide rapidly
- Can be utilized for both transient and stable expression
- Can be cultured in suspension or as a monolayer
- Scalable to higher volumes

And most significantly, their ability to produce fully human post-translational modifications.

The most recent example of usage of HEK293 platform for vaccine production is, COVISHIELD™, a COVID-19 vaccine containing recombinant SARS-CoV-2 spike (S) glycoprotein. This vaccine has been manufactured using genetically modified HEK293 cells.

All the HEK-based vaccine manufacturing processes require a highly efficient and productive serum free and animal component free medium at each step starting from cell banking to cell expansion and virus production in bioreactor.

HEKin1™ is a serum free and animal component free medium optimized for the growth and expansion of HEK293 cells under serum-free conditions. This is a complete media that will support growth of HEK293 cells without further supplementation. This media has been tested for its ability to support high-density cultures of HEK293 cells.

- Scalability for use in shake flasks & bioreactors
- Completely defined system eliminates variability
- Consistent performance improves reproducibility
- Decreased possibility of contamination by adventitious agents
- Saves time with simplified purification and downstream processing
- Supports high cell density, culture longevity and increased yield
- Manufactured in GMP & ISO 9001 certified facility

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HEKin1™

For Suspension Culture

Product Code : SFM039AP

Product Specifications

Part	Name	Storage & Shipping temperature	Available Pack Sizes	
A	Basal Medium	2-8°C	10L	50L
B	Lyophilized Growth supplement		10L	5x10L

Intended use

Intended for *in vitro* research and manufacturing processes only. Do not use for injection or infusion.

Product Performance

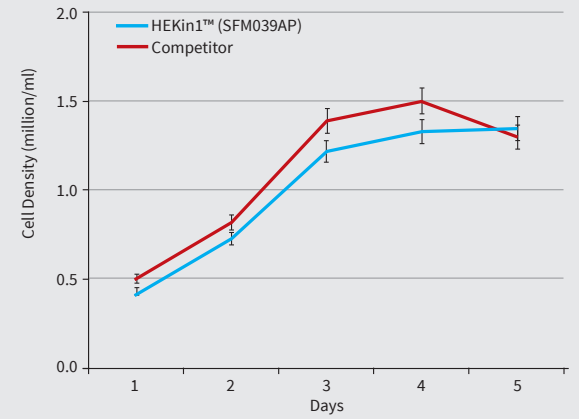

Fig 1 : HEK293 cells adapted to suspension culture were inoculated in SFM039AP & Competitor medium, in 50ml bioreactor tubes with the density of 0.5 x 10^6 cells/ml. Cells were cultured at 37°C, 5% CO_2, for 5 days

Scalability

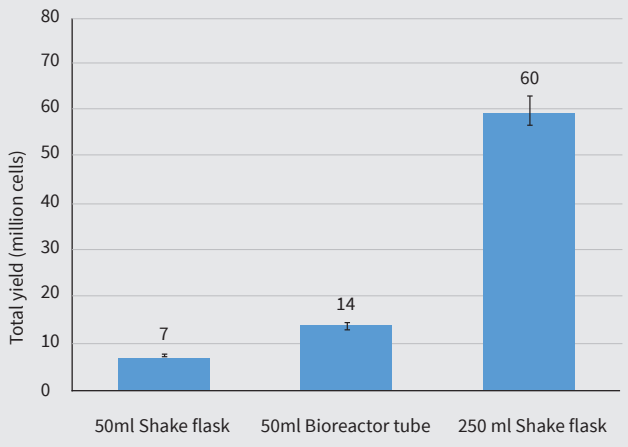

Fig 2 : Scalability of HEK293 suspension cells in HEKin1™ (SFM039AP) from 50ml to 250ml Shake flas

Directions for Use

Preparation of complete medium

This medium does not require supplementation with Pluronic F-68®

Ingredient	SFM039AP
Part A	16.8g in 900ml cell culture grade water
Sodium bicarbonate	3.1g sodium bicarbonate powder
L-Glutamine	876 mg L-Glutamine powder
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 7.1
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

Test	SFM039AP
Appearance	Part A: White to off-white homogenous powder Part B: White coloured lyophilized powder
Solubility	Clear solution at 16.8 g/L
pH without sodium bicarbonate	5.90 – 6.50
pH with sodium bicarbonate	7.50 – 8.10
Osmolality without sodium bicarbonate	290 – 330mOsm/KgH_2O
Osmolality with sodium bicarbonate	320 – 380mOsm/KgH_2O
Cultural response	The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells from morphology and quantitatively by estimating cell counts.
Endotoxin content	NMT 1EU/ml

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HEKin1™

For Adherent Culture

Product Code : SFM038AP

Product Specifications

Part	Name	Storage & Shipping temperature	Available Pack Sizes	
A	Basal Medium	2-8°C	10L	50L
B	Lyophilized Growth supplement		10L	5x10L

Intended use

Intended for in vitro research and manufacturing processes only. Do not use for injection or infusion.

Product Performance

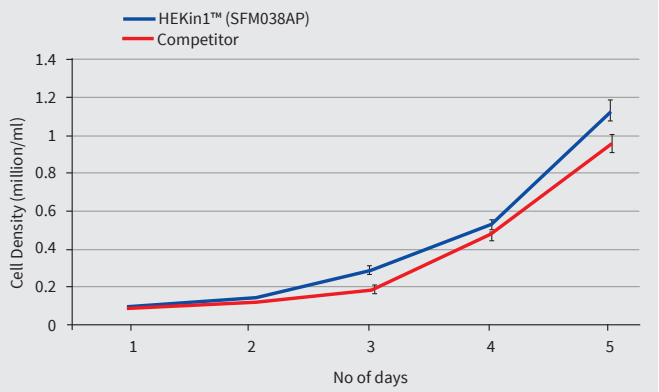

Fig 3 : HEK293 cells adapted to adherent culture were inoculated in SFM038AP & competitor medium, in surface treated T12.5 flasks. Cells we e cultured at 37°C, 5% CO_2 for 5 subcultures.

Scalability

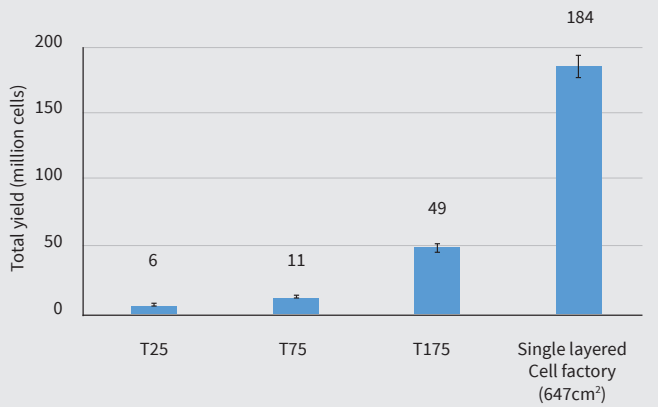

Fig 4: Scalability of HEK293 adherent cells in HEKin1™ (SFM038AP) from T25 flask o single layered cell factory

Directions for Use

Preparation of complete medium

This medium does not require supplementation with Pluronic F-68®

Ingredient	SFM038AP
Part A	13.3g in 900ml cell culture grade water
Sodium bicarbonate	3.1g sodium bicarbonate powder
L-Glutamine	876mg L-Glutamine powder
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 7.1
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 addition	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

Test	SFM038AP
Appearance	Part A: White to off-white homogenous powder Part B: White coloured lyophilized powder
Solubility	Clear solution at 13.3 g/L
pH without sodium bicarbonate	4.40 – 4.80
pH with sodium bicarbonate	7.00 – 7.60
Osmolality without sodium bicarbonate	250 – 290mOsm/KgH_2O
Osmolality with sodium bicarbonate	340 – 380mOsm/KgH_2O
Cultural response	The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells from morphology and quantitatively by estimating cell counts.
Endotoxin content	NMT 1EU/ml

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Procedure for Adaptation

Critical points

Cells used for adaptation should exhibit healthy morphology and have more than 90% viability. Cells should be in the mid-logarithmic phase of growth. It is necessary to 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.

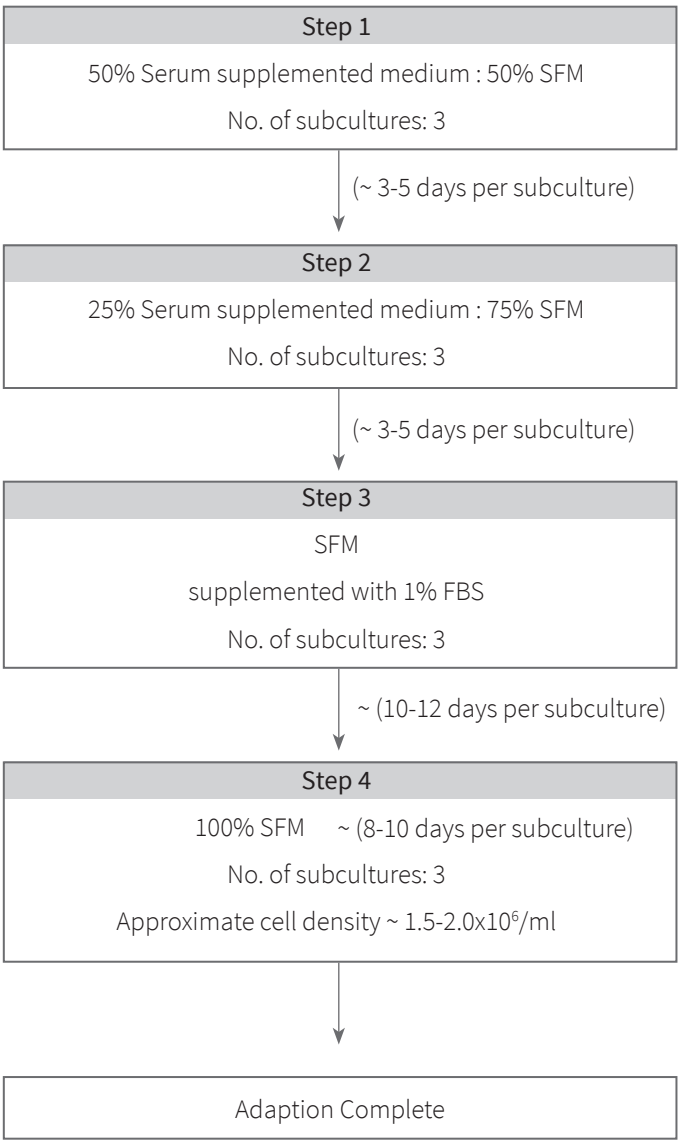
In our experience, HEK293 adherent cells require gradual weaning for adaptation from serum- containing to serum-free conditions. Once adapted, the serum-free adherent culture can be directly adapted to serum-free suspension culture.

Gradual weaning

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.

This procedure is applicable for adaptation of HEK293 adherent cells from serum containing conditions to serum –free conditions.

1. Subculture the cells from serum containing medium **(STEP 1)** and seed them in a 50:50 ratio of serum containing medium and SFM with a seeding density of 0.1-0.2X10^6 cells/ml.
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 Trypsin-EDTA solution.
4. Repeat steps 1 to 3 for three subcultures of each step of gradual adaptation.
5. Determine cell density and reseed the cells in 25:75 ratio of serum containing medium and SFM038AP **(STEP 2)**.
6. After step 2 (25:75 serum containing medium: SFM) of adaptation, the cells cannot be directly sub-cultured 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 in SFM supplemented with 1% serum **(STEP 3)**, before serum free conditions.
7. When the cells reach 100% serum free step **(STEP 4)** of adaptation, subculture them till the cell density of 1.5-2.0X10^6 cells/ml is obtained within 8-10 days of culture. At this point, the cells are considered to be adapted to serum free conditions.



Note: The timelines mentioned above are based on in-house experiments for gradual adaptation. They may slightly vary depending on experimental conditions.

Direct Adaptation

This procedure is applicable for adaptation of HEK293 cells from existing serum free medium (Adherent and Suspension) to HEK293 serum-free HEKin1™ medium (Adherent and Suspension).

Cell harvesting from existing serum-free medium	Harvest the cells from existing serum-free medium in log phase of growth	
Centrifugation	Transfer the entire content to a centrifuge tube and centrifuge for 3 minutes at 1000 rpm.	
Cell density and Viability	Count the cells using hemocytometer, and make a note of cell count and viability.	
Resuspension	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.	
	SFM038AP (Adherent)	**SFM039AP (Suspension)**
Reseeding	Seed with 0.2 × 10^6/ml density in a new cell culture flask containing fresh HEKin1™ (SFM038AP) complete medium	Seed with 0.5 × 10^6/ml density in a new cell culture flask containing fresh HEKin1™ (SFM039AP) complete medium.
Incubation	Incubate the cells at 37°C and 5% CO_2 in static mode	These cells cannot be directly shifted to shaker condition. Incubate the cells at 37°C and 5% CO_2 in **static mode** for 3 sub-cultures.
Sub-culturing	Monitor the cell health every day. Sub-culture once the cells reach 70-80% confluence by trypsinization. Neutralize the trypsin by adding equal volume of trypsin inhibitor. *Note: Change the medium alternate day until the cells reach 70-80% confluen e. When seeded with 0.2× 10^6 cells/ml, 1.5- 2× 10^6 cells/ml are obtained a er subculturing*	Monitor the cells health under the microscope. Determine cell density and viability every day. Sub-culture when the density is double the seeding density. *Note: When seeded with 0.5× 10^6 cells/ml, 1.5- 2× 10^6 cells/ml are obtained a er subculturing*
Maintenance	Continue maintenance of these cells in static conditions for 3 passages	Continue maintenance of these cells in static conditions for total 3 passages. After 3 passages, these cells can be seeded with 0.5 million cells/ml density in a new shake flask containing fresh HEKin1™ (SFM039AP) complete medium. *Note: Cells should have more than 70% viability while being shi ed to shaker condition.*
Incubation	—	For shaker condition, maintain the flasks at 37°C, 5% CO_2 at 120 rpm.
Completion of Adaptation	Cells are considered to be fully adapted to HEKin1™ (SFM038AP) on completion of 3 passages and they can be used for further applications.	Cells are considered to be fully adapted to HEKin1™ (SFM039AP) on completion of 3 passages in shaker condition and they can be used for further applications.

DOs and DON'Ts

- Do not use trypsin and inhibitor for SFM039AP
- Do not refrigerate cells after splitting, seed immediately.
- Vigorous pipetting will stress the cells and loss of viability.
- Do not allow cells to reach 100% confluency before sub-culture.
- Lower seeding densities may cause loss of cell viability.

Routine maintenance of adapted cells

HEK293 cells adapted to serum-free medium can be maintained in both the static culture & suspension culture.

Static Culture

For adherent culture

1. Seed adapted HEK293 cells in T-Flask at $0.1x10^6$/ml density.
2. Incubate at 37°C, 5% CO_2.
3. Monitor the cell heath every day. Subculture once the cells reach 70-80% confluence by trypsinization. Neutralize the trypsin by adding equal volume of trypsin inhibitor.
4. Centrifuge the cells at 1000rpm. Discard the spent medium.
5. Re-suspend the pellet in fresh medium & re-seed in new vessel at $0.1x10^6$/ml density.

Shaker Culture

For suspension culture

1. Seed the adapted cells into shaker flask of required volume with seeding density of $0.5x10^6$/ml.
2. Incubate at 37°C, 5% CO_2 & 120 rpm.
3. Determine the cell density every day & subculture them when the density reaches $1.5\text{-}2.0x10^6$ cells/ml.
4. Centrifuge the cells at 1000rpm. Discard the spent medium.
5. Re-suspend the pellet in fresh medium & re-seed in new vessel at $0.5x10^6$/ml density.

Bioreactor Cultivation

Users are recommended to optimize incubation density, DO, agitation & other parameters empirically, depending on bioreactor scale. Supplementation with Pluronic F68 is not required in bioreactor.

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 complete medium is 4-6 weeks at 2-8°C.

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

CORPORATE OFFICE
HiMedia Laboratories Pvt. Ltd., Plot No. C40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (West) 400604, Maharashtra, India. Tel No.: 022-69034800.
Email: atc@himedialabs.com. Website: www.himedialabs.com.