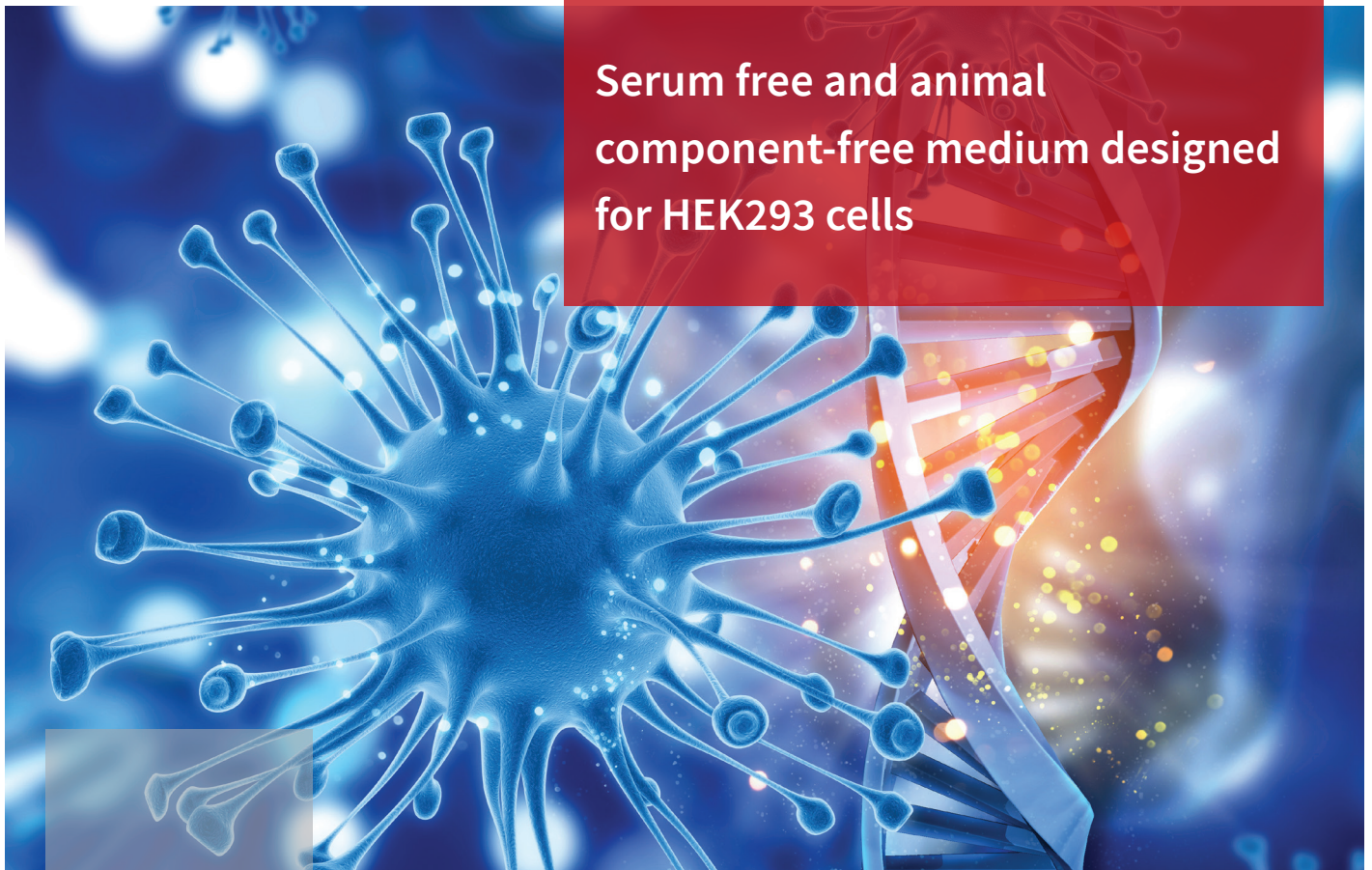


HEKin1™

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



HIMEDIA®

For Life is Precious

CELLBIO™

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
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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
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L-Glutamine (From non-animal source) Cell Culture Tested	TC243-10G TC243-25G
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L-Glutamine 200mM Solution L-Glutamine in 0.85% normal saline Cell culture tested	TCL012-20ML TCL012-100ML
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Sodium bicarbonate powder (Sodium hydrogen carbonate) Cell culture tested	TC230-100G TC230-500G
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7.5% Sodium bicarbonate solution	TCL013-100ML TCL013-500ML
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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
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Trypsin Inhibitor from Soyabean 1X w/ 1mg/ml of Trypsin inhibitor in Dulbecco's Phosphate Buffered Saline	TCL068-100ML
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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

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



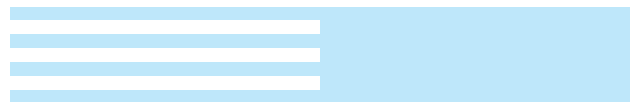
Saves time with simplified purification and downstream processing



Supports high cell density, culture longevity and increased yield



Manufactured in GMP & ISO 9001 certified facility



HEKin1™

For Suspension Culture

Product Code : SFM039AP

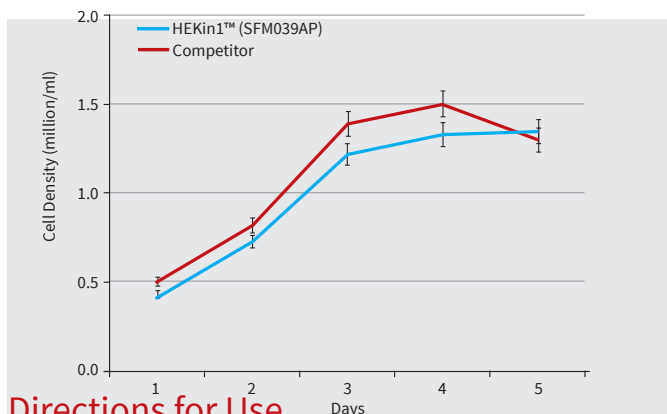
Product Specifications

Part	Name	Storage & Shipping temperature	Available Pack Sizes	
A	Basal Medium	2-8°C	10L	50L
B	Frozen Growth supplement	-20°C	10L	5x10L

Intended use

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

Product Performance



Directions for Use

Fig 1 : HEK293 cells adapted to suspension culture were inoculated in SFM039AP & Competitor medium, in 50ml bioreactor tubes with the density of 0.5×10^6 cells/ml. Cells were cultured at 37°C, 5% CO₂, for 5 days

Scalability

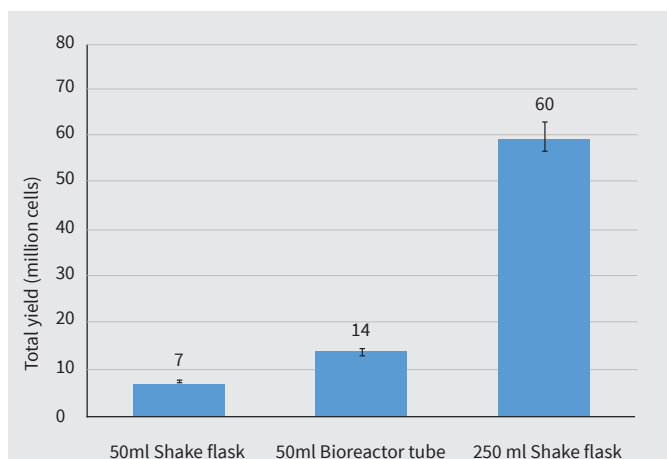


Fig 2 : Scalability of HEK293 suspension cells in HEKin1™ (SFM039AP) from 50ml to 250ml Shake flask

Preparation of complete medium

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

Ingredient	SFM039AP
Part A	14.0g 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	<ul style="list-style-type: none"> Thaw the growth supplement (Part B) overnight at 2-8°C Mix gently by pipetting up and down
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: Pale yellow colored clear solution
Solubility	Clear solution at 14.0 g/L
pH without sodium bicarbonate	5.90 – 6.50
pH with sodium bicarbonate	7.20 – 7.80
Osmolality without sodium bicarbonate	210 – 250mOsm/KgH ₂ O
Osmolality with sodium bicarbonate	280 – 320mOsm/KgH ₂ O
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

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	Frozen Growth supplement	-20°C	10L	5x10L

Intended use

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

Product Performance

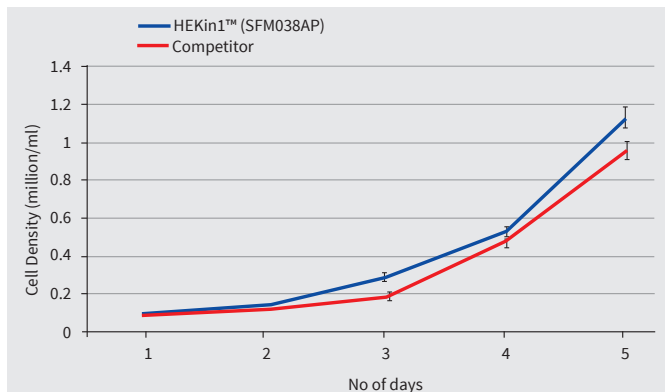


Fig 3 : HEK293 cells adapted to adherent culture were inoculated in SFM038AP & competitor medium, in surface treated T12.5 flasks. Cells were cultured at 37°C, 5% CO₂ for 5 subcultures.

Scalability

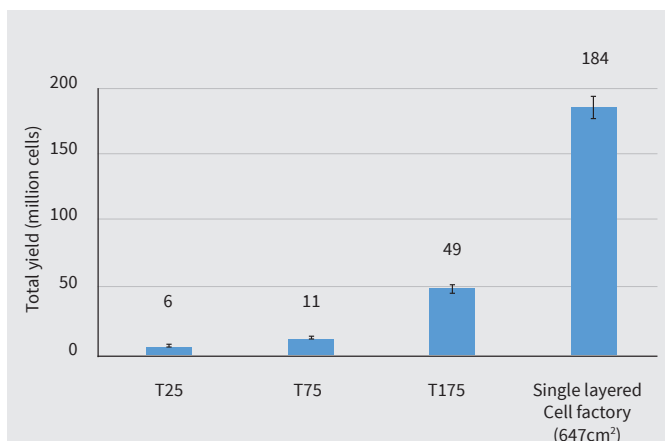


Fig 4: Scalability of HEK293 adherent cells in HEKin1™ (SFM038AP) from T25 flask to single layered cell factory

Directions for Use

Preparation of complete medium

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

Ingredient	SFM038AP
Part A	13.0g 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	<ul style="list-style-type: none">Thaw the growth supplement (Part B) overnight at 2-8°CMix gently by pipetting up and down
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: Clear colorless solution
Solubility	Clear solution at 13.0 g/L
pH without sodium bicarbonate	4.40 – 4.80
pH with sodium bicarbonate	7.40 – 8.00
Osmolality without sodium bicarbonate	240 – 280mOsm/KgH ₂ O
Osmolality with sodium bicarbonate	300 – 340mOsm/KgH ₂ O
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

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 0.1×10^6 /ml density.
2. Incubate at 37°C, 5% CO₂.
3. Monitor the cell health 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 0.1×10^6 /ml density.

Shaker Culture

For suspension culture

1. Seed the adapted cells into shaker flask of required volume with seeding density of 0.5×10^6 /ml.
2. Incubate at 37°C, 5% CO₂ & 120 rpm.
3. Determine the cell density every day & subculture them when the density reaches $1.5-2.0 \times 10^6$ 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 0.5×10^6 /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 -20°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.



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Annexure I: Adaptation Procedure for HEKin1

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

	Gradual Adaptation	
Definition	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	SFM038AP ~ 0.3 million cells for one flask	SFM039AP ~ 15 million cells for one flask
Culture vessel	T- flasks (12.5cm²) - 30 Nos (2 flasks per step X 3 passages = 6 flasks per step)	Shake flasks (125ml) - 30 Nos (2 flasks per step X 3 passages = 6 flasks per step)
Volume of complete media	400 - 500ml*	

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

Gradual / Sequential adaptation

Recommended for adaptation of cells from

- Serum-containing medium to HEKin1™ Serum Free Medium
- Existing serum free medium to HEKin1™ Serum Free Medium

Step	% of existing SFM	% of HEKin1	Seeding density (x 10 ⁶ cells/ml)	No of passages at each stage*	Critical check points before each subculture	Acceptance criteria
1	75	25	For SFM038AP – 0.1 For SFM039AP – 0.5	2-3	1. Morphology	Healthy
2	50	50			2. Viability	More than 90%
3	25	75			3. Time between two passages	3 – 5 days
4	10	90			4. Cell density before passaging	2X – 3X of the seeding density = 1 to 1.5 million cells/ml
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

The procedure and anticipated outcomes mentioned above are based on the results on in-house adaptation experimentation

Technical Support

For any queries / support at any stage of the adaptation, contact us on below mentioned details –

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