

## Instructions For Use HEKstar 293 Media System

### Introduction

HEKstar 293 is a chemically defined, protein-free and animal component-free expression medium developed specifically for stable protein production in HEK293 cells.

HEKstar 293 enables reliable suspension adaptation of various HEK293 derivatives, supports stable growth kinetics with viabilities consistently above 90%, and delivers high protein yields from development to large-scale bioreactor production. Due to its broad formulation, besides stable protein expression, HEKstar 293 is suitable for a wide range of different HEK production processes including pilot scale transient transfection workflows.

### HEKstar 293 Application Overview

Feature	HEK293 expression in suspension cultures	Suitability
Expression System	Stable (fed)-batch expression	+++
	Transient expression	+
Product of Interest	Protein production	+++
	Viral vector production	++
	Virus production	++
Suitable Cell Lines	Most HEK293 cell lines and derivatives in suspension	

### Components of the HEKstar 293 Media System

The HEKstar 293 Media System consists of an expression medium and two feeding supplements. Feeding strategies and cell clone-specific supplementation should be evaluated and optimized during process development prior to large-scale production to achieve optimal performance.

### Components of the HEKstar 293 Media System

Product Name	Formulation	Volume	Cat No.
HEKstar 293 Chemically Defined, Protein-free Expression Medium with L-Glutamine	with 8mM L-Glutamine with 7.0 g/L Glucose with 0.1% Pluronic™	500 ml	STAR-500ML
HEKstar 293 Feed A Chemically Defined Feeding Supplement	with 90.0 g/L Glucose with 0.1% Pluronic™ without L-Glutamine	100 ml	STARFA-100ML
HEKstar 293 Feed B Chemically Defined Feeding Supplement	without Glucose without Pluronic™ without L-Glutamine	10 ml	STARFB-10ML
HEKstar 293 Expression Kit 1 x 500 ml HEKstar 293 Expression Medium (STAR-500ML) 2 x 100 ml Feed A (STARFA-100ML) 2 x 10 ml Feed B (STARFB-10ML)	(see above)	Kit	STAR-K1

### Supplements

Glucose Solution (250 g/L)	50 ml	GLC-F
L-Glutamine (200 mM)	100 ml	GLN-B

## Instructions For Use HEKstar 293 Media System

### Adaptation to HEKstar 293 Expression Medium

For robust HEK293 cells grown in a different medium, no adaptation is needed, and cells may be directly transferred into HEKstar 293 Expression Medium. It is advisable to keep a backup culture in the original medium until cells have adapted. For sensitive HEK293 cells, it is possible to observe suboptimal growth after direct adaptation for 3 – 5 passages. In this case sequential adaptation method is recommended. Additional supplements may be added on process and cell line specific considerations.

### Sequential Adaptation

Culture Flask Type	125 ml shake flask
Medium Volume	25 ml
Inoculation Cell Density	0.8 – 1.2 x 10 <sup>6</sup> cells/ml
Shaking Rate (Orbital Shaker)	110 rpm (50 mm amplitude) 130 rpm (10 mm amplitude)
Temperature	37°C
CO <sub>2</sub> Concentration	5.0%

- Subculture cells into 6.25 ml of supplemented HEKstar 293 Expression Medium mixed with 18.75 ml of the original medium (1:4 ratio). During the adaptation procedure, seed at twice the normal seeding density. Subculture cells when confluency reaches 70 – 90%.
- Once consistent cell growth with high viability has been achieved, passage cells into fresh medium with an increased concentration of HEKstar 293 Expression Medium. Perform adaptation using the following mixture compositions:

Step	Ratio	Volume HEKstar 293 (ml)	Volume Original Medium (ml)
1	1:4	6.25	18.75
2	1:2	12.5	12.5
3	3:4	18.75	6.25
4	9:10	22.5	2.5
5	1:1	25	0

Multiple passages at each step of adaptation may be needed.

- Continue to monitor and passage cells until consistent growth with high viability is achieved. After several passages in 100% new medium, the culture is adapted.

Cell adaptation is strongly recommended before performing expression experiments in batch or fed-batch reactors.

### Cryopreservation

HEK293 cells can be preserved in HEKstar293 Expression Medium supplemented with 7% DMSO or in DMSO-free cryopreservation media such as FreezeMe ZERO (Cat. No. FMZ-50ML), which support long-term storage and maintenance of genetic integrity. For cryopreservation, the HEK293 cells should be in the mid-log phase of growth with a viability of >90%.

#### Cryopreservation

1. Collect the cells in a sterile tube and centrifuge for 5 min at 190 x g
2. Carefully remove the supernatant, resuspend the HEK293 cells to a density of 2.5 – 3.5 x 10<sup>7</sup> cells/ml in the cryopreservation medium.
3. Aliquot the cell suspension to a freezing volume of 0.5 – 1.5 ml. If freezing in larger volumes such as bags is intended, please use programmed cooling curves.

## Instructions For Use HEKstar 293 Media System

- Place the aliquots to a -80°C freezer or use an automated controlled rate freezing supply (a temperature decrease of 0.5°C – 1°C per minute is recommended).
- Transfer the aliquots to liquid nitrogen for long-term storage

### Cell Thawing and Culturing

#### Thawing & Culturing of cryopreserved HEK293 cells

Culture Flask Type	125 ml shake flask
Medium Volume	25 ml
Inoculation Cell Density	0.8 – 1.2 x 10 <sup>6</sup> cells/ml (Thawing) 0.5 – 1.0 x 10 <sup>6</sup> cells/ml (Subculturing)
Shaking Rate (Orbital Shaker)	110 rpm (50 mm amplitude) 130 rpm (10 mm amplitude)
Temperature	37°C
CO <sub>2</sub> Concentration	5.0%

#### Cell thawing

- Remove a vial of HEK293 cells from liquid nitrogen and thaw the cells rapidly in a 37°C water bath. Transfer the vial to a laminar hood as soon as the vial is thawed or with small ice crystals.
- Add 1 ml of the cell suspension to a reaction tube with 10 ml cold HEKstar 293 Expression Medium and centrifuge the cells at 190 xg for 5 min at 4°C to remove the DMSO and other components of the cryopreservation medium.
- Remove the supernatant from the reaction tube using a 10 ml serological pipette without touching the cell pellet. Take out as much liquid as possible in order to remove traces of DMSO without destroying the pellet.
- Resuspend the pellet in pre-warmed HEKstar 293 Expression Medium and set to a viable cell density of **0.8 – 1.2 x 10<sup>6</sup>** cells/ml to a final volume of 25 ml in a 125 ml shake flask.
- Incubate in a humidified incubator at 37°C, 5% CO<sub>2</sub> at 110-130 rpm.  
**Important Note:** Allow freshly thawed cells to recover in culture for two or more passages post-thaw before production.

#### Subculturing

- Prior to subculturing determine the viability and growth rate. The viability should be >90% and the HEK293 cells in the mid-logarithmic growth phase.
- Dilute the HEK293 cells to a viable cell density of 0.5 – 1.0 x 10<sup>6</sup> cells/ml in prewarmed HEKstar 293 expression medium and incubate in a humidified incubator at 37°C, 5% CO<sub>2</sub> at 110 – 130 rpm.
- For subsequent routine cell culture maintenance, subculture cells every two days.

### Fed-Batch Expression with HEKstar 293

#### Recommended Conditions for Initial Testing

Culture Flask Type	250 ml
Medium Volume	50 ml
Inoculation Cell Density	0.8 – 1.2 x 10 <sup>6</sup> cells/ml
Shaking Rate (Orbital Shaker)	110 rpm (50 mm amplitude) 130 rpm (10 mm amplitude)
Temperature	37°C
CO <sub>2</sub> Concentration	5.0%

## Instructions For Use HEKstar 293 Media System

### Initial Feeding Strategy

Add feeding supplements according to the table below. Feed volume is a percentage [%] of initial culture volume at day 0. For example, 3 % feeding volume for a 50 ml culture corresponds to 1.5 ml Feeding supplement.

Culture days	0	1	2	3	4	5	6	7	8	9	10	11
HEKstar Feed A to add [% in v/v]				3	3	3	3	3	3	3	3	3
HEKstar Feed B to add [% in v/v]				0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

### Important Notes

- Never supplement Feed A & Feed B at the same time
- Harvest cells, when viable cell density falls below 60%
- Glucose level should be maintained at 6.0 g/L
- Residual glucose in the culture should not sink below 2.0 g/L
- Depending on productivity and cell viability, feeding schedule and concentration should be adapted

### Transfection with HEKstar 293

HEKstar 293 is optimized for stable fed-batch production and is suitable for pilot-scale testing and in-process controls in transient transfection applications where high yield is not critical.

### Recommended Conditions for Pilot Scale Transfections

Culture Flask Type	250 ml
Medium Volume	50 ml
Inoculation Cell Density	1 × 10 <sup>6</sup> viable cells/ml at day 0 2 – 4 × 10 <sup>6</sup> viable cells/ml at day 2 (transfection)
Shaking Rate (Orbital Shaker)	110 rpm (50 mm amplitude) 130 rpm (10 mm amplitude)
Temperature	37°C
CO <sub>2</sub> Concentration	5.0%
DNA Concentration	1.5 µg/ml
PEI Concentration	2.0 – 4.0 µg/ml

### Protocol for Transfection

#### Day 0: Cell Passaging

1. Determine the viable cell density of the HEK293 cells in an exponential growing culture.
2. Dilute the cells in prewarmed HEKstar 293 Expression Medium to a viable cell density of 1 × 10<sup>6</sup> viable cells/ml. *Note: The inoculated flask will be used for transfection, so prepare a separate culture flask for stock cultures.*
3. Incubate the cells in a 37°C incubator with 5% CO<sub>2</sub> in a humidified environment on a shaker for two – three days.

#### Day 2: Transfection of Cells

1. Two days later, recheck the viable cell density and dilute the cells to a viable cell density of 2 – 4 × 10<sup>6</sup> viable cells/ml in prewarmed HEKstar 293 Expression Medium.
2. Keep the cells in a 37°C incubator with 5% CO<sub>2</sub> on a shaker while preparing the DNA/transfection complex.

## Instructions For Use HEKstar 293 Media System

3. The volume of the DNA:PEI-complex should be 3 – 5% of the total working volume (e.g., the DNA:PEI complex should have a volume of 1.5 – 2.5 ml for a total culture volume of 50 ml)
4. Dilute PEI transfection reagent to a concentration of 2.0 – 4.0 µg/ml in DMEM Medium to 50% of the total DNA:PEI complex volume (e.g., 0.75 – 1.25 ml for a total culture volume of 50 ml). Important Note: The ideal concentration of PEI should be optimized according to cell line and process specific considerations.
5. Mix gently and incubate for 5 – 10 min at room temperature.
6. In parallel, dilute DNA to a concentration of 1.5 µg/ml in DMEM Medium to 50% of the total DNA:PEI complex volume (e.g., 0.75 – 1.25 ml for a total culture volume of 50 ml).
7. Mix gently and incubate for 5 – 10 min at room temperature.
8. Add the diluted DNA to the diluted PEI, mix gently and incubate for 10 min at room temperature.
9. Add the DNA:PEI complex to the freshly diluted cells and mix gently.
10. Incubate the cells in a 37°C incubator with 5% CO<sub>2</sub> in a humidified environment at 110 – 130 rpm.
11. After 24 hours, add 5% HEKstar 293 Feed A and 0.5% HEKstar 293 Feed B of the total culture volume.
12. Incubate in a 37°C incubator with 5% CO<sub>2</sub> in a humidified environment at 110 – 130 rpm until harvest.

### Precautions and Disclaimer

---

This product is for research use and further manufacturing only.  
Pluronic is a trademark of BASF Corporation.

### Help Needed?

---

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).