

ExCell Bio

OptiViro[®] 293 Serum-free Medium VirTrans HE03

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	HE000-N071
Catalog Number	HE000-N072
Catalog Number	HE000-N081
Catalog Number	HE000-N082
Catalog Number	HE000-N083
Catalog Number	HE000-N084



| PRODUCT DESCRIPTION

OptiVibro® 293 Serum-free Medium VirTrans HE03 is a chemically-defined medium that does not contain any animal-derived components. It is specifically designed for expanding HEK293-derived suspension cell lines such as 293T and 293F cells. This medium allows for efficient growth and transfection of multiple suspension 293 cell lines, with a significant increase in AAV titer when combined with OptiVibro® 293 Serum-free Feed Medium HA03. Manufactured in accordance with Good Manufacturing Practices (GMP) guidelines and free of antibiotics.

| SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Name	Cat.#	Specification	Storage	Transportation	Shelf Life
OptiVibro® 293 Serum-free Medium VirTrans HE03	HE000-N071	500 mL	Store at 2-8°C. Protect From Light.	<25°C, Protect From Light.	12 months
	HE000-N072	1000 mL	Store at 2-8°C. Protect From Light.	<25°C, Protect From Light.	12 months
OptiVibro® 293 Serum-free Medium VirTrans HE03(Powder)	HE000-N081	1 L	Store at 2-8°C. Dark and dry.	<10°C, Protect From Light	24 months
	HE000-N082	10 L	Store at 2-8°C. Dark and dry.	<10°C, Protect From Light	24 months
	HE000-N083	100 L	Store at 2-8°C. Dark and dry.	<10°C, Protect From Light	24 months
	HE000-N084	500 L	Store at 2-8°C. Dark and dry.	<10°C, Protect From Light	24 months

| PERFORMANCE, APPLICATION AND HANDLING

RECOMMENDATIONS

1. Store cell culture medium in a dark environment, ideally in colored packaging, to protect it from light exposure.
2. Avoid prolonged exposure to fluorescent or other types of lighting during transport to prevent discoloration.
3. Implement thorough cleaning and sterilization methods for transport to sterile areas; avoid UV sterilization.
4. Switch off UV lamps when transferring through UV-sterilized windows.

| INSTRUCTION FOR USE

Medium preparation

To prepare 1L of liquid medium from OptiVibro® 293 Serum-free Medium VirTrans HE03 (Powder) (ExCell Bio, HE000-N08#), follow these steps:

1. Start with a clean vessel and add approximately 800 mL of water.
2. Slowly add 24.032 g of medium powder while stirring continuously. Mix for about 30-40 minutes.
3. Adjust the pH with 5 mol/L NaOH to 8.5-8.8. Mix for 10 minutes.
4. Slowly add 2.317 g of sodium bicarbonate powder and mix for 10 minutes.
5. Adjust the pH to 6.9-7.2 with 6 mol/L HCl solution. Mix for another 10 minutes.
6. Add water to reach a final volume of 1L, and continue stirring for an additional 5 minutes.
7. Sterilize by 0.22 µm PES membrane filtration.
8. Store the medium in a cool, dark place at 2°C to 8°C for up to 12 months.

Cell Culture

1. Incubate cells at 37°C in a humidified atmosphere with 5-8% CO₂, using an orbital shaker platform rotating at either 125 rpm (with a 19 mm orbital diameter) or 95 rpm (with a 50 mm orbital diameter).

2. To culture 293 cells in a shake flask, seed a suspension of cells at a concentration of $0.3-1 \times 10^6$ cells/mL, with a recommended culture volume of 20-30 mL in a 125 mL shake flask. Subculture cells every 48-72 hours or when cell density reaches $4.0-6.0 \times 10^6$ cells/mL.

3. If cells have just been recovered from a frozen state or cultured in other brands of medium, subculture them for three passages before use.

Recommendation of Transfection

1. Following cell recovery, subculture cells consistently at least three times to ensure cell viability exceeds 90%.

2. The day prior to transfection, seed cells at an inoculation density of 1.1×10^6 cells/mL in fresh medium.

3. On the day of transfection, adjust the cell volume to 18 mL with fresh medium, maintaining a final transfection density of around 2.2×10^6 cells/mL.

4. **Prepare PEI/DNA complex:** This protocol details the transfection process with a culture volume of 20 mL, a cell density of 2×10^6 cells/mL, a DNA concentration of 1.5 µg/mL, and a DNA:PEI ratio of 1:3.

1) Dilute 90 µg of PEI Max with 1 mL of OptiVibro® 293 Serum-free Medium VirTrans HE03, incubate the mixture at room temperature for 5 minutes.

2) Dilute 30 µg of DNA with 1 mL of OptiVibro® 293 Serum-free Medium VirTrans HE03, incubate the mixture at room temperature for 5 minutes.

3) Add the PEI Max solution to the DNA solution to create the PEI/DNA complex, thoroughly mix the solutions and incubate at room temperature for an additional 10 minutes to allow the complex to form.

5. Gently introduce 2 mL of the PEI/DNA complex into the cell suspension, ensuring thorough mixing.

6. After 18-24 hours post-transfection, add 5% volume of OptiVibro® 293 Serum-free Feed Medium HA03.

7. Typically, the culture is harvested on day 2 post-transfection.

If larger volumes of cell transfection are needed, the recommended amount of the reagents are listed below:

Table 1. Recommended dosage for various transfection specifications

Cell culture vessel	125 mL	500 mL	1 L	Remark
Amount of cell ($\times 10^6$ cells)	40	200	400	cell density 2×10^6 cells/mL
OptiViro® 293 Serum-free Medium VirTrans HE03 (mL)	18	90	180	Initial culture volume
DNA diluent (mL)	1	5	10	
PEI diluent (mL)	1	5	10	
DNA (μ g)	30	150	300	DNA: PEI=1:3
PEI Max (μ g)	90	450	900	
OptiViro® 293 Serum-free Feed Medium HA03 (mL)	1	5	10	5% of the initial transfection volume
Final culture system (mL)	~21	~105	~210	/

Table 2. Related products

Product Name	Cat.#	Specification
OptiViro® 293 Serum-free Feed Medium HA02	HA000-N031	100 mL Liquid
	HA000-N032	1000 mL Liquid
	HA000-N041	1 L Powder
	HA000-N042	10 L Powder
	HA000-N043	50 L Powder

【Note】

1. The inoculation density is designed to achieve a transfection density of about 2.2×10^6 cells/mL, and can be adjusted based on the cell expansion rate. Adjusting the seed density by fresh medium dilution may decrease protein titer.
2. The provided transfection techniques are informational; a Design of Experiments (DOE) approach can be utilized to establish optimal experimental design.
3. The timing of feeding and harvesting may vary and can be optimized based on project requirements.

| DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk, and our company will not be responsible for any resulting product performance deviations.
2. This product is for scientific research and commercial production only and is not intended for clinical diagnosis or treatment. Users assume all risks for unauthorized use, and our company shall not be responsible for any consequences.