EIV22024E

ExCell Bio

OptiVitro® 293 Serum-free Feed Medium HA03

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	HA000-N031S
Catalog Number	HA000-N031
Catalog Number	HA000-N032
Catalog Number	HA000-N041
Catalog Number	HA000-N042
Catalog Number	HA000-N043

PRODUCT DESCRIPTION

OptiVitro® 293 Serum-free Feed Medium HA03 is a chemically-defined medium that does not contain any animal-derived components, which can lead to a significant increase in AAV titer. Manufactured in accordance with Good Manufacturing Practices (GMP) guidelines and free of antibiotics, this medium is designed to support the growth and productivity of HEK293-derived suspension cell lines, particularly in the context of AAV production.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Name	Cat.#	Specification	Storage	Transportation	Shelf Life
OptiVitro® 293 Serum-free Feed Medium HA03	HA000-N031S	50 mL	Store at 2-8°C. Protect From Light.	<10°C， Protect From Light.	12 months
	HA000-N031	100 mL	Store at 2-8°C. Protect From Light.	<10°C， Protect From Light.	12 months
	HA000-N032	1000mL	Store at 2-8°C. Protect From Light.	<10°C， Protect From Light.	12 months
OptiVitro® 293 Serum-free Feed Medium HA03(Powder)	HA000-N041	1 L	Store at 2-8°C. Dark and dry.	<10°C， Protect From Light.	24 months
	HA000-N042	10 L	Store at 2-8°C. Dark and dry.	<10°C， Protect From Light.	24 months
	HA000-N043	50 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 OptiVitro® 293 Serum-free Feed Medium HA03 (Powder), follow these steps:

1. Start with a clean vessel and add approximately 600 mL of water.

2. Slowly add 153.85 g of OptiVitro® 293 Serum-free Feed Medium HA03 (Powder) to the water while stirring continuously. Mix for about 60 minutes.

3. Adjust the pH with 5 mol/L NaOH to 8.5 ~ 8.8 (about 45 mL). Mix for 60 minutes.

4. Adjust the pH to 6.9-7.2 with 6 mol/L HCl solution (about 10 mL). Mix for another 10 minutes.

5. Add water to reach a final volume of 1L, and continue stirring for an additional 5 minutes.

6. Sterilize by 0.22 μm PES membrane filtration.

7. 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% CO_2, 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 0.3-1×10^6 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 4.0-6.0×10^6 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 OptiVitro® 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 OptiVitro® 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 OptiVitro® 293 Serum-free Feed Medium HA03.

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 (×10^6 cells)	40	200	400	cell density 2×10^6 cells/mL
OptiVitro® 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 (μg)	30	150	300	DNA: PEI=1:3

PEI Max (μg)	90	450	900	
OptiVitro® 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
OptiVitro® 293 Serum-free Medium VirTrans HE03	HE000-N072	1000 mL Liquid
OptiVitro® 293 Serum-free Medium VirTrans HE03 (Powder)	HE000-N081	1 L Powder
	HE000-N082	10 L Powder
	HE000-N083	100 L Powder
	HE000-N084	500 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.