EIV32025E

ExCell Bio

OptiVitro® 293 Serum-free Medium VirTrans HE03

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

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

PRODUCT DESCRIPTION

OptiVitro® 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 OptiVitro® 293 Serum-free Feed Medium HA03.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

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

HANDLING RECOMMENDATIONS

1. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. During the transportation of the product, it should be kept away from light. This is to prevent the product from being affected by the irradiation of fluorescent lamps or other light sources, which may lead to discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning process. The cleaning method may involve disinfectant wiping, and not utilize UV irradiation.

   Note: *When passing through transfer windows equipped with UV lamp, remember to proactively turn off the UV lamp inside the transfer window.*

Medium preparation

Instructions for preparing 1L of OptiVitro® Serum-free Medium VirTrans HE03 with the powder:

1. Measure 80% of the final required volume of WFI or cell culture grade water in a clean vessel.
2. Slowly add 24.032 g of OptiVitro® Serum-free Medium VirTrans HE03 (powder) while stirring continuously. Mix for about 30-40 minutes.
3. Slowly add approximately 4.5 mL of 5 mol/L NaOH solution, adjust the pH to 8.5-8.8, then stir for 10 minutes.
4. Slowly add 2.317 g of sodium bicarbonate powder and mix for 10 minutes.
5. Slowly add approximately 3.5 mL of 6 mol/L HCl solution, adjust the pH to 7.0-7.2, then stir for 10 minutes.
6. Add water to reach a final volume of 1L and continue stir for an additional 5 minutes.
7. Measure and record the final pH and osmolality. The pH should be 6.9-7.5, osmolality should be 290-330 mOsm/kg.
8. Sterilize by 0.22 μm PES membrane filtration, store the medium at 2-8°C, protect from light.

Cell Culture

1. Culture 293 cells at 37°C in a humidified atmosphere with 5% CO_2, 90-120 rpm. According to the condition of cell growth, pass cells every 48-72 hours or when cell density reaches 4.0-6.0×10^6 cells/mL. The suggested seeding density is 0.6-1.0×10^6 cells/mL. Extra Glutamine is not needed for the culture.
2. If 293 cells were originally cultured in another brand's medium, they can be directly or gradually transferred to this medium for passage culture. After three passages (9-10 days), the cells will adapt to the new medium, achieving stable proliferation and viability, and can then be used for subsequent experiments.
3. If 293 cells were frozen after being cultured in another brand's medium, it is recommended to first thaw them using the original medium. After one passage, switch to this medium. Following three additional passages, when proliferation and viability have stabilized, the cells can be used for experiments. Cells that were frozen in this medium can be directly thawed and cultured in it.

Recommendation of Transfection

Below is an introduction to the usage of OptiVitro® 293 Serum-free Medium VirTrans HE03 and OptiVitro® 293 Serum-free Feed Medium HA03. They can be used in combination to achieve higher virus titer.

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 the density of 1.1×10^6 cells/mL in fresh medium.

Note: *This seeding density is designed to achieve a density of about 2.2×10^6 cells/mL at the day of transfection, and can be adjusted based on the cell expansion rate.*

3. On the day of transfection, adjust the cell volume to 18 mL with fresh medium, in a 125 mL shake flask culture system (20 mL working volume). The total cell count should be 4.0×10^7 cells, achieving a transfection cell density of approximately 2.2×10^6 cells/mL.
4. Prepare PEI/DNA complex:

This protocol is optimized for the transfection process with a culture volume of 20 mL, a cell density of 2.0 ×10^6 cells/mL, a DNA concentration of 1.5 μg/mL, and a DNA:PEI ratio of 1:3.

PEI MAX solution: dilute 90 μg of PEI MAX (Polysciences#24765-1) with 1 mL of OptiVitro® 293 Serum-free Medium VirTrans HE03, incubate the mixture at room temperature for 5 minutes.

DNA solution: 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.

PEI and DNA combination: 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. Slowly add the prepared 2 mL PEI/DNA complex to the culture system, then transfer to the incubator for continued cultivation.
6. Around 24 hours after transfection, add 5% volume of OptiVitro® 293 Serum-free Feed Medium HA03. For every 20 mL culture volume, add 1 mL of the feed medium.
7. Typically, the culture is harvested on 48-72 hours post-transfection.
8. If larger volumes of cell transfection are needed, the recommended amount of the reagents are listed below in Table 1. To achieve optimal virus packaging titer, it is recommended to use our feed medium in combination. For details, refer to Table 2.

Table 1. Recommended dosage for various transfection specifications

Cell culture vessel	125 mL	500 mL	1 L
Amount of cell (×10^6 cells)	40	200	400
OptiVitro® 293 Serum-free Medium VirTrans HE03 (mL)	18	90	180
DNA diluent (mL)	1	5	10
PEI diluent (mL)	1	5	10
DNA (μg)	30	150	300
PEI MAX (μg)	90	450	900
OptiVitro® 293 Serum-free Feed Medium HA03 (mL)	1	5	10
Final culture system (mL)	~21	~105	~210

Table 2. Related products

Product Name	**Cat. #**	**Specification**
OptiVitro® 293 Serum-free Feed Medium HA03	HA000-N031	100 mL Liquid
	HA000-N032	1000 mL Liquid
OptiVitro® 293 Serum-free Feed Medium HA03 (powder)	HA000-N041	1 L Powder
	HA000-N042	10 L Powder

Note:

1) The provided transfection techniques are informational; a Design of Experiments (DOE) approach can be utilized to establish optimal experimental design.

2) *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.