SARTORIUS

Simplifying Progress

Novel Chemically Defined, Animal Component Free Medium for Vero Cells and Virus Production for Human and Animal Vaccines

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Introduction

Vero cells are a lineage of cells isolated from the kidney of African green monkey by Yasumura and Kawakita in Japan (1962). Vero cells are used for various purposes, most importantly for the production of cell culture-based viral vaccines. The cell line is among a very limited list of cell lines that have been approved by health authorities for the production of human vaccines. Vero cells are increasingly used in the production of approved human vaccines protecting against viral infections such as rabies, rotavirus, Japanese Encephalitis and Poliomyelitis. When scale-up is required, Vero cells can be cultured on micro-carrier beads in bioreactors using the appropriate culture medium. Currently available media contain a vast amount of undefined polypeptides or animal derived proteins that may result in batch to batch variations, as well as an increased potential for contamination with adventitious agents and therefore increase the risk for safety issues.

NutriVero™ Flex 10 is a chemically defined, serum-free, animal-component free medium optimized for both 2D monolayer and 3D microcarriers suspension cultures, and is suitable for a wide range of applications, from large scale cell culturing to virus production. This medium properties assure consistent results and maximum control over your virus production process.

Abbreviations

ACF	Animal Component Free	PI	Post Infection
CD	Chemically Defined	SF	Serum Free
CPE	Cytopathic Effect	XF	Xeno Free

Materials and Methods

Cell culture
Vero cells were cultured in NutriVero™ Flex 10 (BI, Catalog No. 05-068-1), as well as a commercially available animal component free (ACF) culture media in both 2D and 3D culture systems.
2D culture system – included static T-flasks and culture dishes. Vero cells were detached using Recombinant Trypsin EDTA (BI, Catalog No. 03-079-1) and counted using Nucleocounter NC-3000 (Chemometec).
3D culture systems – Cytodex-1 Microcarriers (GE, Catalog No. 17-0448-01) were used in spinner flasks or bioreactors. The bioreactors were filled up to 2 L of working volume of tested medium. Stirring speed was set between 70 and 130 rpm, temperature set to 37°C and pH controlled to 7.2. The bioreactors were seeded with 0.15 × 10^9 cells/L and 3g/L of Cytodex-1.

Powder media version
The powder version of NutriVero™ Flex 10 Base was dissolved with purified water, stirred at RT for 30 minutes and supplemented with the required supplements. Vero cells were cultured in this medium at the same conditions as the liquid medium, in both 2D and 3D culture systems.

Virus production
The initial assessment of viral production was performed in 2D culture system infected with various viruses. Next phase included a 3D culture system in bioreactor. Following 72 hrs of culture, Vero cells reached a concentration of approximately 1 × 10^6 cells/ml, and then the viruses were added. The virus production was assessed by Cytopathic Effect (CPE) using a light microscopy and measuring virus titers.

Soy hydrolysate
0.1% soy hydrolysate (Kerry, Catalog No. HY PEP 5603N) was added to the medium and the different parameters (cell concentration and virus yield) were measured.

Results – Vero Cell Growth

Vero cell growth and density in 2D and 3D culture systems
NutriVero™ Flex 10 was tested in a defined ACF system for cell growth and cell density. Utilizing a 2D culture system, NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extract containing medium (VP-SFM).

When tested using Cytodex-1 microcarriers in a 2 L bioreactor, Vero cells were adhered to the microcarriers 24 hrs following seeding, and after 120 hrs all microcarriers were fully confluent with cells homogeneously distributed. NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extract containing medium (VP-SFM).

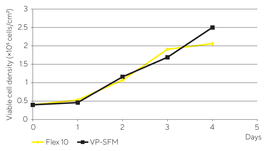

Figure 1: Growth curve of Vero cells in 2D culture system.

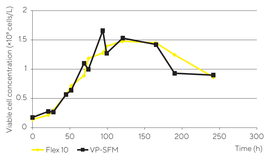

Figure 2: Growth curve of Vero cells in 3D culture system (bioreactor).

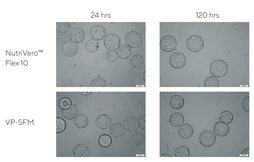

Figure 3: Representative images of 3D culture system on Cytodex-1 microcarriers.

Results – Virus Production

Virus production in 2D and 3D culture systems
Initial assessment of defined NutriVero™ Flex 10 viral production capacity was performed using a 2D culture system and infection by various viruses. Virus titer for NutriVero™ Flex 10 was comparable to undefined medium (VP-SFM).

For virus production assessment in 3D culture system, Vero cells were cultured in bioreactors and infected with various viruses. Both culture duration up to complete CPE, as well as virus titers using NutriVero™ Flex 10 were found to be better than an undefined control medium (VP-SFM).

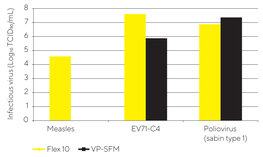

Figure 4: Virus production in 2D culture system.

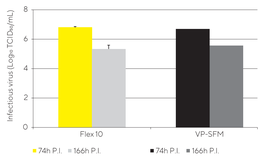

Figure 5: Virus production (EV71-C4) in 3D culture system.

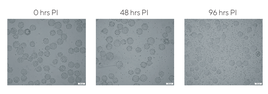

Figure 6: Representative images of Vero cells grown in 3D culture system and infected with Sabin poliovirus type 3.

Results – Soy Hydrolysate

Insignificant effect of soy hydrolysate
To assess the effect of plant hydrolysate on the performance of defined NutriVero™ Flex 10, 0.1% soy hydrolysate was added to the medium. In both 2D and 3D culture systems the addition of plant hydrolysate had no effect on Vero cell growth.

For virus production assessment, Vero cells were seeded in a 2D culture system and infected with various viruses. The addition of hydrolysate did not significantly enhance the production of any of the viruses.

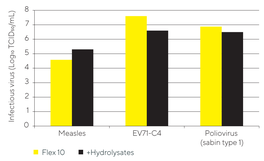

Figure 7: Virus production in 2D culture system with or without plant hydrolysates.

Results – Powder NutriVero™ Flex 10

Powder version of NutriVero™ Flex 10
NutriVero™ Flex 10 powder medium was assessed in comparison to the liquid form of the media using 2D and 3D culture systems. Vero cells growth was comparable in both NutriVero™ Flex 10 versions.

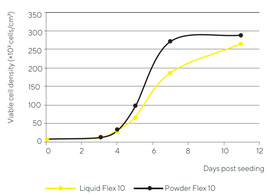

Figure 8: Growth curve of Vero cells in 2D culture system.

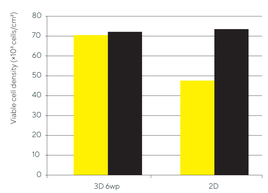

Figure 9: Cell count of Vero cells on day 5 in 3D vs. 2D culture systems.

Results – Stability

NutriVero™ Flex 10 liquid medium stability
NutriVero™ Flex 10 stability was assessed up to 18 months post production. Utilizing a 2D and 3D systems, Vero cells were seeded in NutriVero™ Flex 10 defined medium and counted using Nucleocounter NC-3000 (Chemometec) at indicated time points.

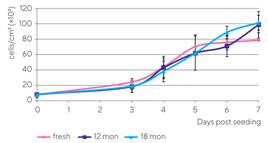

Figure 10: Growth curve of Vero cells in 2D culture system.

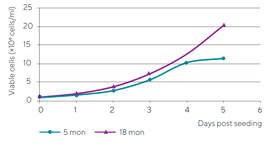

Figure 11: Growth curve of Vero cells in 3D culture system (bioreactor).

Summary

Chemically defined, serum-free, animal-component free NutriVero™ Flex 10 medium demonstrated excellent results in Vero cell growth and virus yield in both 2D and 3D culture systems.

Containing solely recombinant components and no plant hydrolysates, NutriVero™ Flex 10 showed equal performance and in some cases was found to be superior to commercially available undefined medium. Furthermore, the addition of plant hydrolysate to NutriVero™ Flex 10 did not enhance cell growth and virus yield.

NutriVero™ Flex 10 liquid medium showed good results with shelf life of 18 months. Utilizing NutriVero™ Flex 10 removes variability that is in correlation with undefined extracts thus reducing regulatory and health safety concerns as well as manufacturing costs. Its excellent performance enables a reliable and safe defined ACF system in vaccine manufacturing.