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Biological Industries
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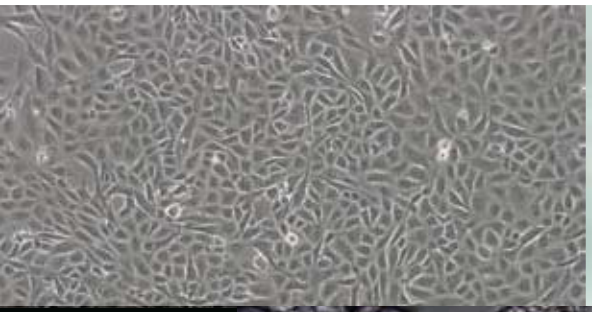
# NutriVero™ Flex 10



Next-generation chemically defined serum-free, animal component free medium designed to support the growth of Vero cells

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# Introduction

Isolated from the kidney of the African green monkey by Yasumura and Kawakita in Japan (1962), the Vero cell line is used for various purposes, most importantly for the production of cell culture-based viral vaccines. Reasons for the extensive use of the Vero cell line are the consistent high viral yields and relatively easy adaptation for growth in bioreactors on microcarriers, thus allowing greater vaccine purity and as well as quantity[1].

The Vero cell line is among a very limited list of cell lines that have been approved by health authorities worldwide for the production of human vaccines and is very well-documented on performance in quality and quantity of viral yield.

Currently available media contain a vast amount of undefined polypeptides or animal derived raw materials that may result in lot-to-lot variations, inconsistency as well as an increased potential for contamination with harmful agents and therefore increased risk for safety issues.

To reduce manufacturing costs and improve health safety, it is crucial to move towards a chemically defined cell culture media for vaccine manufacturing.

NutriVero™ Flex 10 is a chemically defined, serum-free, animal component-free medium, newly developed to support Vero cell growth in monolayers as well as in microcarriers suspension culture systems and optimized for the production of viruses. Defined NutriVero™ Flex 10 is a robust, customizable medium which contains solely recombinant components and does not contain any plant extract (hydrolysates), therefore providing consistent results which are difficult to obtain with an undefined medium.

Defined NutriVero™ Flex 10 is 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. Developed together with Intravacc, an R&D organization for translational vaccinology, this chemically defined, serum-free, animal component free medium will give you consistent results and maximum control over your virus production process.

## NutriVero™ Flex 10 Features

- Suitable for 2D and 3D cultures
- Chemically defined – no plant or animal components
- Very low protein concentration
- Lot-to-lot consistency
- Reduced risk of contamination
- Optimal for the production of various viruses (incl. measles, enterovirus, polio)
- Suitable for direct adaptation
- Easy downstream product purification
- Produced in a cGMP-compliant facility in a xeno-free dedicated production line.

| Product Name | Cat. No. | Size | Storage |
|---|---|---|---|
| NutriVero™ Flex 10 | 05-068-1A | 500ml | +4°C |

## Minimize regulatory concerns

Animal-derived components (e.g. fetal bovine serum and chicken embryos) have been a part of the viral vaccine manufacturing process for decades. The development of serum-free cell culture systems allowed to progress to a more reliable and safer manufacturing process. Yet, most of the serum-free, animal component-free media used today in the vaccine manufacturing industry are based on plant-based hydrolysates, which are inconsistent and undefined.
Culturing Vero cells in defined NutriVero™ Flex 10 eliminates the risks associated with the use of animal-origin components and removes variability that is in correlation with undefined extracts. Utilizing a completely defined medium significantly reduces regulatory concerns in human vaccine production without compromising on performance, quality and quantity of viral yield. This enhancement can result in more efficient manufacturing and the desirable outcome of lower production costs.

Defined NutriVero™ Flex 10 provides the ultimate environment for improved cell viability and yield, as well as high virus production, while maintaining a complete defined animal component-free manufacturing process.

**NutriVero™ Flex 10**

**From small scale research to large scale production**

## Scale-up possibilities

When scale-up is required, Vero cells can be cultured in a 2D system (i.e. roller bottles) or preferably in a 3D system using microcarrier beads in spinner flasks, shake flasks or bioreactors. Utilizing microcarriers in your culture system greatly enlarges the available surface area, thus increasing the number of cells, virus titer and providing efficient large-scale production.
Cytodex (GE Healthcare) microcarriers are used for many applications in the vaccine industry (i.e. influenza vaccine and large scale Vero cell production) and allow cultivation of large quantities of viruses (i.e. Adeno, Bovine Rhinotrachteitis, Measles, Rous Sarcoma, Endogenous C-type, Papova, Rubella Equine Rhinopneumonitis, Parvo, Sendai, Foot-and-Mouth, Polio, Influenza, Rabies)[2].
A full validation of NutriVero™ Flex 10 medium has been performed on 3D cultures using Cytodex-1 microcarriers.

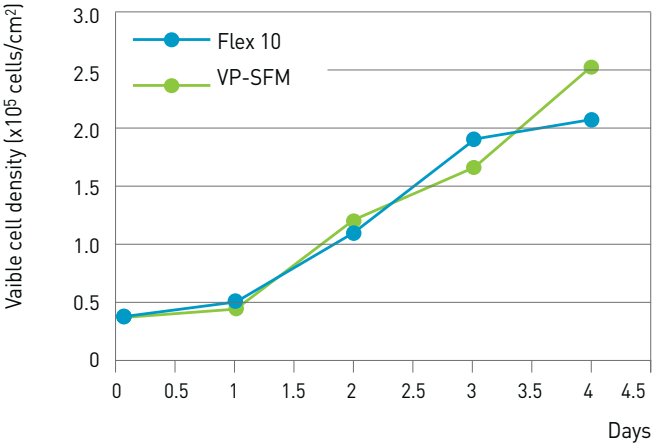
# Technical Data

## Vero cell growth and cell density in 2D and 3D systems

### Vero cell growth and cell density in a 2D culture system

Defined NutriVero™ Flex 10 was tested in a defined animal-component free system for cell growth and cell density. Utilizing a 2D culture system, NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extracts containing medium (VP-SFM) (figure 1).

**Figure 1:** Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm$^2$ and incubated at 37°C in a humidified atmosphere and 5% $CO_2$ with NutriVero™ Flex 10 or VP-SFM.
At indicated times post seeding cells were harvested and re-suspended in medium for cell count and viability using a nucleocounter N-100.




### Vero cell growth and cell density in a 3D microcarrier culture system

Defined NutriVero™ Flex 10 was tested using Cytodex-1 microcarriers in a 2L bioreactor to assess Vero cell growth curve and cell density under controlled conditions.
Vero cells were adhered to the microcarriers 24hrs following seeding, and after 120hrs all microcarriers were fully confluent with cells homogeneously distributed (figure 2). NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extracts containing medium (VP-SFM) (figure 3).

**Figure 2:** Vero cell growth on microcarriers in 2L stirred tank bioreactor at 24 and 120 hours.
Microphotographs of representative cell culture in defined NutriVero™ Flex 10 and undefined medium (VP-SFM).

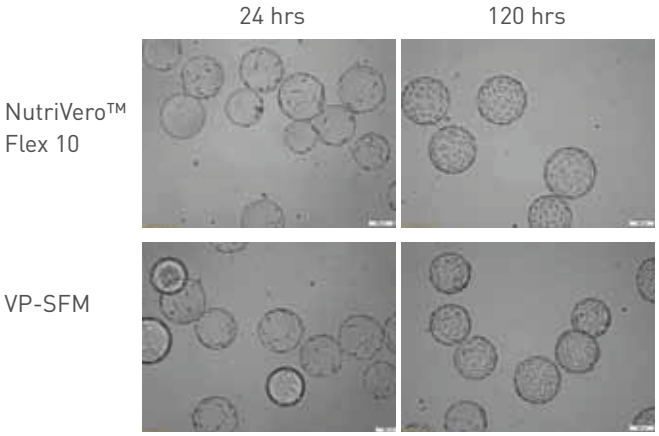
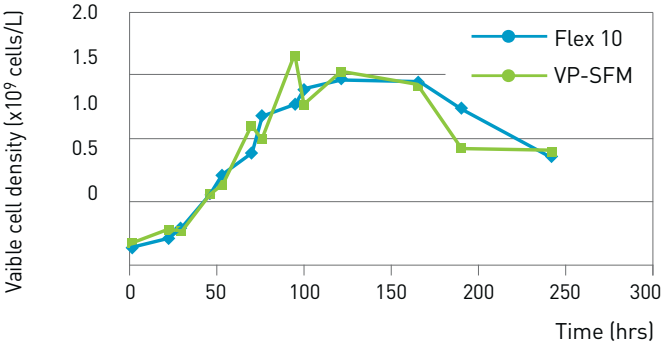



**Figure 3:** Two parallel bioreactors were filled up to 2L of working volume of defined NutriVero™ Flex 10 and undefined medium (VP-SFM). Stirring speed was set between 70 and 130 rpm, temperature set to 37°C and pH controlled to 7.2. The bioreactors were seeded with 0.15x10$^9$ cells/L and 3g/L of Cytodex-1. At indicated times post seeding, cell suspension was sampled for cell count and viability using a nucleocounter N-100.



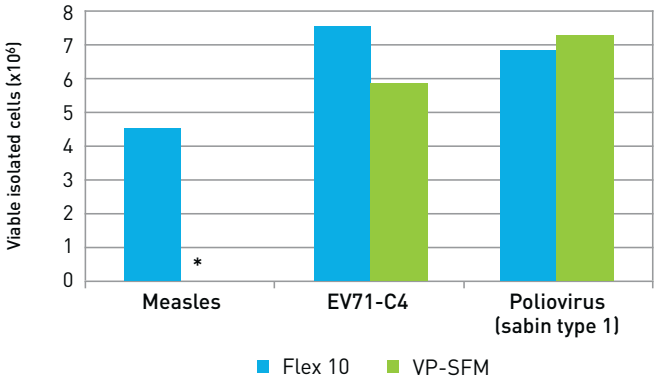
## Virus production in 2D and 3D systems

### Virus production assessment in a 2D culture system

For initial assessment of defined NutriVero™ Flex 10 viral production capacity, Vero cells were seeded in a 2D culture system and infected with various viruses (Figure 4). Virus titer for NutriVero™ Flex 10 was comparable to undefined medium (VP-SFM).

**Figure 4:** Vero cells were seeded in 6-well plates at a cell density of 30,000 cells/cm$^2$ and infected with various viruses. Following 7 days of culture, the cultures showed positive Cytopathic Effect (CPE), and the supernatant was harvested and analyzed for virus titer.

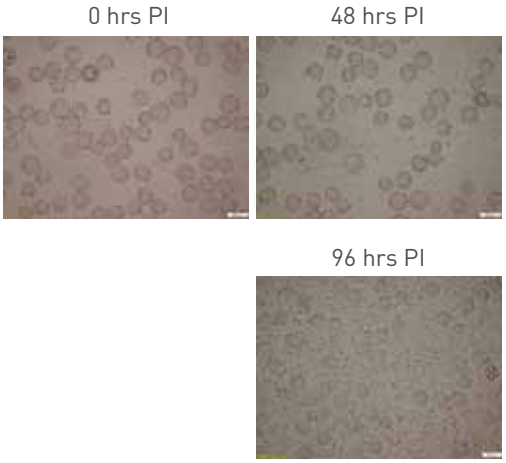
* The virus titers for Measles was below the limit of quantification (LOQ = 4x$\log_{10}$TCID$_{50}$/ml).




### Sabin poliovirus type 3 in a 3D microcarriers culture system

Defined NutriVero™ Flex 10 sustained cell growth in a 2L bioreactor and reached a cell concentration of approximately 1x10$^6$ cells/ml after 72h. All microcarriers appeared homogeneously populated with cells (Figure 5). Upon reaching such concentrations, Sabin poliovirus type 3 was added to the system and CPE was monitored by light microscopy. Defined NutriVero™ Flex 10 did show complete CPE (>95%) at 96h post infection (PI) and all microcarriers appeared deserted.

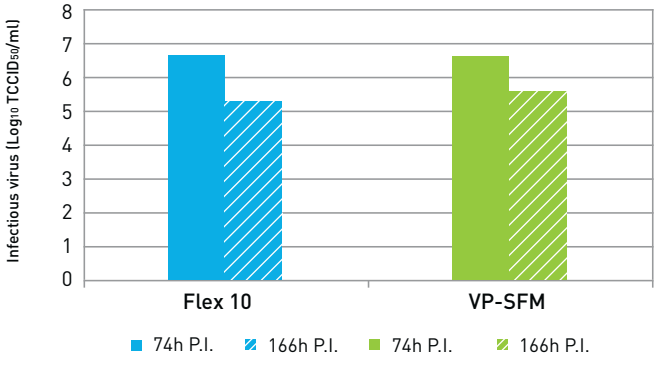
**Figure 5:** Microphotographs of representative cell suspension in NutriVero™ Flex 10 over time.




### Enterovirus 71-C4 virus production in a 3D microcarriers culture system

Defined NutriVero™ Flex 10 was tested in a 1L bioreactor to asses Enterovirus 71-C4 (EV71-C4) production in 3D culture system. Vero cells were cultured in a 1L bioreactor and infected with EV71-C4 virus. Virus titer for defined NutriVero™ Flex 10 was found to be comparable with virus titer of an undefined control medium (VP-SFM) (figure 6).

**Figure 6:** Vero cells were cultured in 1L bioreactors at 0.12±0.03x10$^6$ cells/ml for 3 days, up to a concentration of 0.9±0.2x10$^6$ cells/ml. At 66hrs post seeding, the cells were infected with E71-C4 at a titer of 7.55 $\log_{10}$TCID$_{50}$/ml. Samples were taken at indicated time points post infection, and analyzed for virus titer.

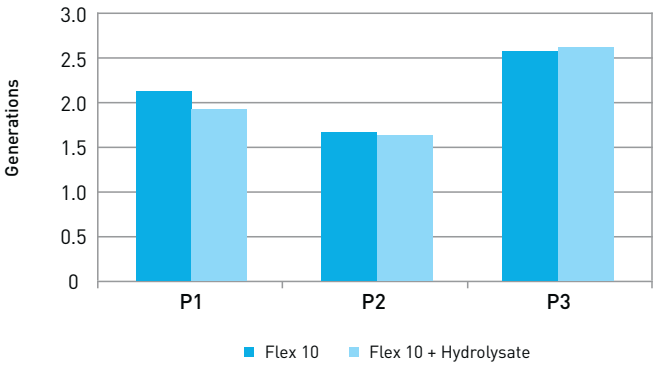


## Insignificant effect of soy hydrolysate on cell growth and virus titer

### Vero cell growth in 2D culture system with the addition of plant 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. Cell concentration was measured upon seeding and during harvesting of 3 passages and the number of generations was calculated. The addition of hydrolysate had no effect on cell growth in 2D culture system.

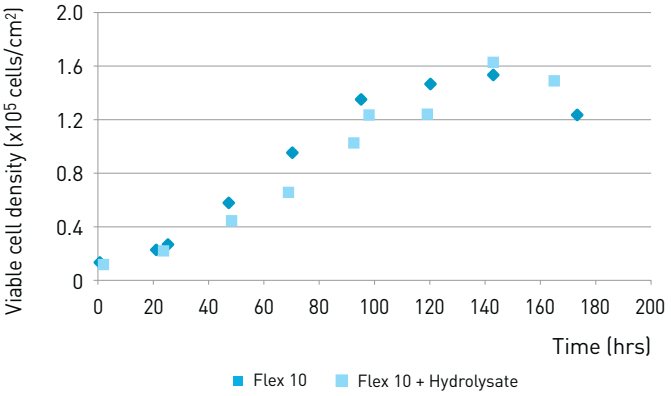
**Figure 7:** Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm$^2$ and incubated at 37°C in humidified atmosphere and 5% $CO_2$.
At each passage (4 days post seeding for passage 1 and 3; 3 days post seeding for passage 2) cells were harvested and re-suspended in medium for cell count and viability using a nucleocounter N-100.




### Vero cell growth in 3D culture system with the addition of plant hydrolysate

The effect of plant hydrolysate was tested in 2L bioreactor under controlled conditions (figure 8). Defined NutriVero™ Flex 10 and NutriVero™ Flex 10 with plant hydrolysate showed similar growth curve.

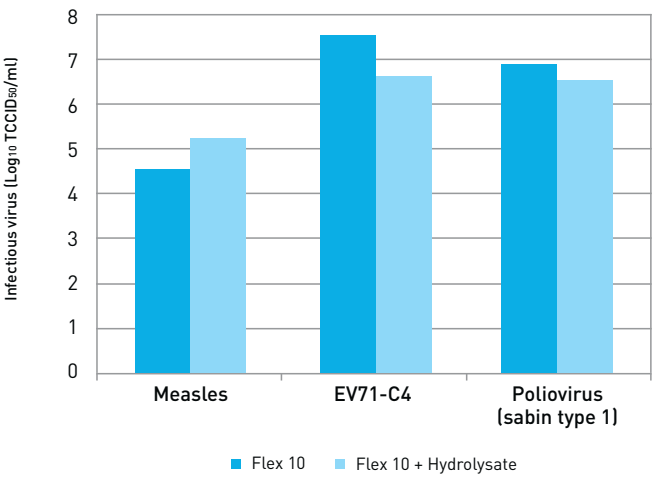
**Figure 8:** Two 2L bioreactors were filled with defined NutriVero™ Flex 10 and NutriVero™ Flex 10 with 0.1% soy hydrolysate. Stirring speed was set between 70 and 130 rpm, temperature set to 37°C and pH controlled to 7.2. The bioreactors were seeded with 0.15x10$^9$ cells/L and 3g/L of Cytodex-1. At indicated times post seeding, cell suspension was sampled for cell count and viability using a nucleocounter N-100.




### Virus Yield in 3D culture system with the addition of plant hydrolysate

Vero cells were seeded in a 2D culture system and infected with various viruses: Measles, Sabin poliovirus type 1 and EV71-C4. Following 7 days of culture, all cultures showed positive CPE and the supernatant was analyzed for the amount of infectious virus particles (Figure 9). The addition of hydrolysate did not significantly enhance the production of any of the viruses.

**Figure 9:** Vero cells were seeded in 6-well plates at a cell density of 30,000 cells/cm$^2$ of culture, the cultures were infected with various viruses: Measles, Sabin poliovirus type 1 and EV71-C4. Following 7 days the cultures showed a positive CPE and the supernatant was harvested and analyzed for the amount of infectious particles by means of a virus titration procedure.



# Summary

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

Containing solely recombinant components and no plant extracts (no hydrolystates) NutriVero™ Flex 10 shows equal performance and in some cases is superior to undefined competitor medium. Furthermore, the addition of plant hydrolysate to NutriVero™ Flex 10 did not enhance cell growth and virus yield.

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 proofs that utilizing a reliable and safe complete defined system in vaccine manufacturing is now achievable.

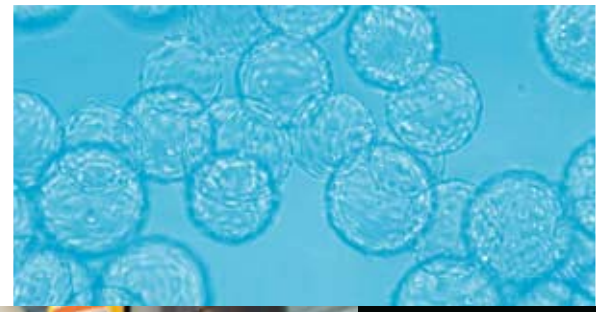
## Auxiliary Products

| Product Name | Cat. No. | Size | Storage |
|---|---|---|---|
| Soybean Trypsin Inhibitor (50X) 5mg/ml | 03-048-1C | 20ml | -20°C |
| Serum-Free Cell Freezing Medium | 05-065-1C<br>05-065-1A | 20ml<br>500ml | 2-8°C |
| Recombinant Trypsin EDTA Solution | 03-079-1B<br>03-079-1C | 100ml<br>20ml | 2-8°C |

**Bibliography**

1. Sheets, R. (2000). History and Characterization of the Vero Cell Line.
2. Microcarrier Cell Culture, principles and methods. GE Healthcare.

* VP-SFM is a trademark of Thermo Fisher Scientific



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