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# NutriVero™ Flex 10 ACF Medium

05-068-1

A Chemically Defined, Animal Component-Free (ACF) Medium, Designed to Support the expansion of Vero Cells and Viral Vaccine Production

# Instructions for Use

## Product Description

NutriVero™ Flex 10 is a chemically defined, animal component-free medium developed to support Vero cell growth in both microcarriers suspensions and monolayer cultures in the areas of virology, virus production, and biotechnology.
There are many problems associated with the use of animal sera, e.g., fear of contamination with adventitious agents such as BSE, Hepatitis, HIV, and BVD. Culturing cells in a serum-free and animal component-free medium eliminates these risks. Furthermore, it allows cells to be grown under a defined set of conditions.
NutriVero™ Flex 10 is appropriate for both microcarriers suspensions and monolayer cultures of Vero cells. NutriVero™ Flex 10 is suitable for large-scale culturing and for virus production as well as other cell culture applications.

**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.

The product is sterile. The sterility is achieved by aseptic manufacturing and filling technique including 0.1µm membrane filtration.

**Precaution and Disclaimer**

1. Do not use if a visible precipitate is observed in the medium.
2. Do not use NutriVero™ Flex 10 Medium beyond the expiration date indicated on the product label.

**Storage and Stability**

NutriVero™ Flex 10 Medium should be stored at 2-8°C. Protect the medium from light.
Avoid head space as it might change the media's pH.
Shelf Life: Refer to product label for expiration date.

## Instructions for Use

**Procedure**
A successful transition from serum-containing media to serum-free media often requires the use of techniques which were specifically developed for this purpose. Special techniques for trypsinization, neutralization of trypsin, cryopreservation of cells, as well as the use of an effective serum-free growth medium are all essential. Careful attention to the details of procedures outlined here are therefore essential in order to guarantee the successful use of NutriVero™ Flex 10.
NutriVero™ Flex 10 is a ready-to-use serum-free medium, which does not require the addition of L-glutamine or Alanyl glutamine. Antibiotics are not specifically recommended, but if required, use a lower concentration than usual.

**Adaptation of the cells to NutriVero™ Flex 10**
In most cases it is possible to seed cells that have been removed from freezing medium directly into NutriVero™ Flex 10, when the cell concentration is approximately $8x10^3$ cells per $cm^2$. The cells will begin to grow in NutriVero™ Flex 10, and after a few passages the adaptation will be complete.
Cells should be passaged for a minimum of three passages in NutriVero™ Flex 10 prior to testing in a growth promotion assay.
After successful adaptation, it is recommended to cryopreserve the cells in NutriFreez™ D10 Cryopreservation Medium (05-713-1) in order to avoid the need for any further adaptation in the future.
For cell dissociation we recommend using Recombinant Trypsin EDTA Solution (Cat. No. 03-079-1), and for trypsin neutralization we recommend using Soybean Trypsin Inhibitor (Cat. No. 03-048-1), which are both chemically defined reagents and contain no products of animal origin.

**Adaptation from serum-containing media:**
If adapting directly from a serum-supplemented medium to NutriVero™ Flex 10, the initial cell plating density should be greater than that used with serum-containing media for the first 2-3 passages.
In cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with NutriVero™ Flex 10 containing 5% serum, and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case.
In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second passage, it will become evident if direct adaptation has been successful, and if not, only the weaning experiments are continued.

## Quality Assurance

- Manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines.
- Manufactured under controlled environments and processes in accordance with:
  - ISO 13408 – Aseptic Processing of Health Care Products;
  - ISO 14644 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones;

**Product Label Symbols**

| | |
|---|---|
| REF | Indicates the manufacturer's catalogue number so that the medical device can be identified. |
| STERILE A | Indicates a medical device that has been manufactured using accepted aseptic techniques. |

## Culturing Vero cells in NutriVero™ Flex 10

### 1. Recovery of cryopreserved Vero cells

- Pre-warm to room temperature 5-10ml of NutriVero™ Flex 10 in a 50ml conical tissue culture tube.
- Rapidly thaw frozen vial of Vero cells in a 37°C water bath, with gentle agitation until no ice remains.
- Slowly add the cells into the pre-warmed NutriVero™ Flex 10.
- Centrifuge cells at 400g for 4.5 minutes at room temperature.
- Remove supernatant and re-suspend the cell pellet in 0.5-1ml of pre-warmed NutriVero™ Flex 10.
- Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay).
- Add the desired volume of pre-warmed NutriVero™ Flex 10.
- Transfer the cells into desired cultureware. Seeding densities should be calculated (see Table 1).
- Incubate in a humidified $CO_2$ incubator (37°C).

**Recommended seeding densities**
(approximately 8000 cells/cm$^2$)

| Cultureware | Surface area cm$^2$ | Volume of NutriVero™ Flex 10 (ml/well) | Recommended seeding (total cells) |
|---|---|---|---|
| 24-well | 1.9 | 0.5 | 1.52x10$^4$ |
| 12-well | 3.9 | 1 | 3.12x10$^4$ |
| 6-well | 9.6 | 2 | 7.68x10$^4$ |
| T25 | 25 | 5 | 20x10$^4$ |
| T75 | 75 | 10 | 60x10$^4$ |

### 2. Subculturing Vero cells

NutriVero™ Flex 10 Medium was developed for optimal proliferation of Vero cells. We recommend seeding the cells at a concentration of 8000 cells/cm$^2$ (Table 1), re-feeding the cells with fresh warmed NutriVero™ Flex 10 Medium every 2-3 days, and subculturing when the cells reach approximately 80-100% confluence.

**Procedure**

- Remove culture medium and gently wash once with DPBS w/o Ca, Mg (Cat. No. 02-023-1).
- For a T25 culture flask, add 1ml of Recombinant Trypsin Solution with EDTA (Cat. No. 03-079-1) at room temperature (for any other cultureware, the appropriate volume should be adjusted).
- Incubate for 2-4 minutes at 37°C and verify cell detachment using inverted microscope.
- Note: The more the culture is confluent, the slower the detachment will be, and the higher the volume of trypsin recommended
- Following detachment, add 5 ml of pre-warmed Soybean Trypsin Inhibitor (SBTI, Cat. No. 03-048-1, diluted 1:50 in DPBS according to instructions).
- Collect cell suspension into a sterile tube and re-wash the cultureware as necessary to collect the entire cells.
- Centrifuge the cells for 4.5 minutes at 400g at room temperature. Carefully discard the supernatant.
- Re-suspend the cell pellet in minimal volume of pre-warmed NutriVero™ Flex 10 Medium. Take a sample to perform a viable cell count. For cryopreservation continue to Section 3.
- Seed cells into desired cultureware. Seeding densities and the required volume of NutriVero™ Flex 10 Medium to be added should be calculated (see Table 1).
- Incubate in a humidified $CO_2$ incubator (37°C).
- Feed cells with fresh warmed NutriVero™ Flex 10 Medium every 2-3 days.

### 3. Cryopreservation of serum-free cultures

- Grow desired quantity of cells in T-flasks, and harvest when flasks are 80% confluent.
- Following centrifugation, re-suspend the cell pellet in cold NutriFreez™ D10 Cryopreservation Medium at a concentration of 0.5-5x$10^6$ cells/vial, 1ml/vial.
- Freeze the cells gradually (1-2°C/minute using a freezing container [Mr. Frosty™] or controlled cryopreservation system), and store them in liquid nitrogen storage tank (vapor phase).
- Viability and recovery of cryopreserved cells should be checked 24 hours after storage of vials in liquid nitrogen by following the thawing procedure outlined above.

**Spinner Flasks or bioreactors: Microcarrier cultures**
During cell culture the rotational speed of the spinner flask should be 30-60rpm. Seeding densities and attachment conditions for microcarrier cultures should be optimized to provide sufficient time for cell attachment.

**Sample procedure**
Vero cells can be seeded on Cytodex 1 microcarriers in spinner flasks or bioreactors.
For a 250ml spinner flask with a working volume of 100ml, add 3gr/L cytodex 1 and 1x$10^5$ Vero cells/ml and stir at 30rpm.
For 2L bioreactors with a working volume of 2L, add 3gr/L cytodex 1 and 1.3x$10^5$ Vero cells/ml and stir at 100 rpm.

**Quality Control**
The performance of NutriVero™ Flex 10 is tested using Vero cells pre-adapted to serum-free culture in NutriVero™ Flex 10 in 2D and 3D culture systems. Additional standard tests are pH, osmolality, and sterility.

## Auxiliary products

| Product | Cat. No. |
|---|---|
| Recombinant Trypsin EDTA Solution | 03-079-1 |
| Soybean Trypsin Inhibitor (SBTI) | 03-048-1 |
| NutriFreez™ D10 Cryopreservation Medium | 05-713-1 |