

Instructions for Use

4Cell[®] NutriVero[™] Flex 10

A chemically defined (CD), animal component-free (ACF) medium, designed to support the expansion of Vero Cells and viral vaccine production



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1 Introduction

4Cell® 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 certain risks associated with the use of animal sera, e.g., fear of contamination with adventitious agents such as BSE, Hepatitis, and HIV. 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.

4Cell® NutriVero™ Flex 10 is appropriate for both microcarriers suspensions and monolayer cultures of Vero cells. 4Cell® NutriVero™ Flex 10 is suitable for large-scale culturing and for virus production as well as other cell culture applications.

1.1 Intended Use

Not approved for human or veterinary use. **Not** for application in humans or animals, or for use in vitro diagnostic or clinical procedures.

- Suitable for 2D and 3D cultures
- Chemically defined – no animal or plant components
- Very low protein concentration
- Lot-to-lot consistency
- Optimal for the production of various viruses (incl. measles, enterovirus, polio)
- Suitable for direct adaptation
- Easy downstream product purification

1.2 Precaution and Disclaimer

- Do **not** use if a visible precipitate is observed in the medium.
- Do **not** use 4Cell® NutriVero™ Flex 10 Medium beyond the expiration date indicated on the product label.

1.3 Storage and Stability

- 4Cell® NutriVero™ Flex 10 Medium should be stored at 2 – 8°C, and protected from light.
- Shelf Life: refer to product label for expiration date.

2 Instructions for Use

2.1 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 4Cell® NutriVero™ Flex 10.

4Cell® 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.

2.2 Adaptation of the Cells To 4Cell® NutriVero™ Flex 10

In most cases it is possible to seed cells that have been removed from freezing medium directly into 4Cell® NutriVero™ Flex 10, when the cell concentration is approximately 8×10^3 cells per cm^2 . The cells will begin to grow in 4Cell® NutriVero™ Flex 10, and after a few passages the adaptation will be complete.

Cells should be passaged for a minimum of three passages in 4Cell® 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 Soybean Trypsin Inhibitor (Cat. No. 03-048-1), which are both chemically defined reagents and contain no products of animal origin.

2.3 Adaptation From Serum-Containing Media

If adapting directly from a serum-supplemented medium to 4Cell® 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 4Cell® 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.

3 Culturing Vero Cells in 4Cell® NutriVero™ Flex 10

3.1 Recovery of Cryopreserved Vero Cells

NOTE

For the recovery of cryopreserved and subculturing of Vero cells always pre-warm 4Cell® NutriVero™ Flex 10 to room temperature.

- Pre-warm to room temperature 5 – 10 mL of 4Cell® NutriVero™ Flex 10 in a 50 mL 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 4Cell® NutriVero™ Flex 10.
- Centrifuge cells at 400 g for 4.5 minutes at room temperature.
- Remove supernatant and re-suspend the cell pellet in 0.5 – 1 mL of pre-warmed 4Cell® NutriVero™ Flex 10.
- Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay).
- Add the desired volume of pre-warmed 4Cell® NutriVero™ Flex 10.
- Transfer the cells into desired cultureware. Seeding densities should be calculated (see Table 1).
- Incubate in a humidified CO₂ incubator (37°C).

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

Table 1: Recommended seeding densities (approximately 8000 cells/cm²)

3.2 Subculturing Vero Cells

4Cell® NutriVero™ Flex 10 Medium was developed for optimal proliferation of Vero cells. We recommend seeding the cells at a concentration of 8000 cells/cm² (Table 1), re-feeding the cells with fresh warmed to room temperature 4Cell® 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 1 mL 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 400 g at room temperature. Carefully discard the supernatant.
- Re-suspend the cell pellet in minimal volume of pre-warmed 4Cell® 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 4Cell® NutriVero™ Flex 10 Medium to be added should be calculated (see Table 1).
- Incubate in a humidified CO₂ incubator (37°C).
- Feed cells with fresh warmed 4Cell® NutriVero™ Flex 10 Medium every 2 – 3 days.

3.3 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 $0.5 - 5 \times 10^6$ cells/vial, 1 mL/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 – 60 rpm. 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 250 mL spinner flask with a working volume of 100 mL, add 3 gr/L cytodex 1 and 1×10^5 Vero cells/mL and stir at 30 rpm.

For 2L bioreactors with a working volume of 2L, add 3 gr/L cytodex 1 and 1.3×10^5 Vero cells/mL and stir at 100 rpm.

4 NutriVero™ Powder Reconstitution Procedure

1. Measure out 70% of final volume of cell culture grade water / WFI. Water should be at room temperature.
2. While gently stirring, add the powdered medium (13.6gr per liter).
3. Stir at RT for at least 10 minutes or continue until dissolved.
4. Rinse original package with a small amount of water and add to solution.
5. Add 10 mL per liter of supplement (x100 supplement)
6. Add 2,017.3 mg of sodium bicarbonate for each liter of final volume of medium.
7. Stir at RT for at least 10 minutes or continue until dissolved.
8. While stirring, measure pH and adjust to pH 7.2 using NaOH 1N or HCl 1N, if required.
9. Bring medium to final volume with cell culture grade water / WFI.
10. Measure osmolality. Accepted range: 310-330 mOsm/Kg.
11. Filtrate the medium by using 0.1µm PES membrane filter.
12. Aseptically dispense into sterile containers.
13. Store complete medium at 2-8 °C and in the dark.

5 Quality Control



The performance of 4Cell® NutriVero™ Flex 10 is tested using Vero cells pre-adapted to serum-free culture in 4Cell® NutriVero™ Flex 10 in Vero culture systems. Additional standard tests are pH, osmolality, sterility and endotoxins.

For full specifications please check the lot specific Certificate of Analysis (CoA).

6 Quality Assurance

- For research use & further manufacturing
- 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 – Cleanrooms and associated controlled environments

Product Label Symbols

REF	Indicates the manufacturer's catalogue number so that the product can be identified.
LOT	Indicates the manufacturer's batch code so that the batch or lot can be identified. Note: Synonyms for batch code are lot number and batch number.
	Indicates the date after which the product is not to be used.
	Indicates the temperature limits to which the product can be safely exposed.
STERILE A	Indicates a product that has been manufactured using accepted aseptic techniques.

7 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

8 Ordering Information

Product*	Size & Package*	Material No.
4Cell® NutriVero™ Flex 10 Medium P-red	500 mL Bottle	CFV3FA4000
4Cell® NutriVero™ Flex 10 Medium P-red	1L Bottle	CFV3FA4001
4Cell® NutriVero™ Flex 10 Medium	500 mL Bottle	CFV3FA4009
4Cell® NutriVero™ Flex 10 Medium	1L Bottle	CFV3FA4010

* Other sizes are available on request

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Last updated:

07 | 2023

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LM | Publication No.: SCM6023-e230701