BI
Biological Industries

NutriVero VP2 ACF SFM

A chemically defined, animal and human components free serum-free medium, designed to support the growth of Vero cells in the areas of virology, virus production, and biotechnology.

Cat. No.: 05-067-1B 100ml
05-067-1A 500ml

Instructions for Use

Product Description

There are many problems associated with the use of animal sera e.g. the fear of contamination with viral agents such as BSE, Hepatitis, HIV, BVD or other potential adventitious agents. The culture of cells in serum-free and animal component-free medium eliminates those risks. Furthermore, it allows cells to be grown under a defined set of conditions.
NutriVero VP2 is a serum free, very low protein medium containing no proteins or peptides of human or animal origin, designed specifically for microcarriers suspension culture of Vero cells. NutriVero VP2 is suitable for large scale culturing and for growing viruses, as well as other cell culture applications, including production of recombinant proteins. The medium contains EGF and does not contain L- glutamine.

Features
- Very low protein concentration.
- No proteins or peptides of animal or human origin.
- The proteins that are used are human recombinant EGF and human recombinant Insulin.
- The formulation is without any animal origin components.
- Reduced risk of viral contamination.
- Lot to lot consistency.
- Ease of downstream product purification.

Precaution and Disclaime
1. For in vitro diagnostic use.
2. Do not use if a visible precipitate is observed in the medium.
3. Do not use NutriVero VP2 Medium beyond the expiration date indicated on the product label.

Storage and Stability
NutriVero VP2 Medium should be stored at 2°C – 8°C. Protect the medium from light.
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. For example, 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 VP2.
NutriVero VP2 is formulated without human and animal origin components, the trace proteins in NutriVero VP2 are Human recombinant EGF and Human recombinant Insulin. NutriVero

VP2 is a ready-to-use serum-free medium after the addition of 4mM L-glutamine or Alanyl- glutamine. Antibiotics are not specifically recommended but when required, use low concentration then usual. The formulation does not contain attachment factors.

Adaptation of the Cells to NutriVero VP2

In most cases it is possible to seed the cells that have been removed from freezing medium directly in NutriVero VP2, when the cell concentration is at least 5x105 cells per 25cm2. The cells will begin to grow in NutriVero VP2, and after a few passages the adaptation will be complete.

If starting directly from a serum- supplemented medium to NutriVero VP2 the initial cell plating density should be greater than that used with serum-containing media , for the first 2-3 subcultures.

In those 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 VP2 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 be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued.

Cells should be passaged in a minimum of tree passages in NutriVero VP2 perior to testing in a growth performance assay.

After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium (Cat. No. 05-065-1), in order to avoid the necessity of any further adaptation in the future.

It is recommended to use Cell Dissociation Solution which is chemically defined reagent and contains no products of animal origin. If trypsin is used, Crystalline Trypsin Solution is recommended.

Spinner Flasks / Roller Bottle and Microcarriers Culture

Due to the absence of endogenous attachment factors, cell attachment in NutriVero VP2 is slower than in serum-containing media. For this reason the rotational speed of the roller bottles should not exceed 0.3rpm for the first 24 hours. After 24 hours the rotational speed of the roller bottles should be 30-60rpm. Seeding densities and attachment conditions for microcarriers culture should be optimized to provide sufficient time for cell attachment.

Trypsinization with Crystalline Trypsin

Compared to solutions of crude Trypsin, crystalline Trypsin solution does not damage the cells even after prolonged exposure. In addition, the excess Trypsin can be neutralized with SBTI, thus avoiding the introduction of serum. The crystalline Trypsin solution should be thawed, divided into smaller portions and then re-frozen, in order to avoid the necessity of repeated thawing-freezing cycles.

Trypsinization procedure:

1. Enzymatic methods for removing cells from microcarriers are most commonly employed.
2. Stop the stirring and allow the microcarriers to settle.
3. Remove the medium and wash the cells with DPBS solution (without calcium and magnesium) (Cat. No. 02-023-1) for 5 minutes. EDTA at a concentration of 0.02% may be added to the PBS.
4. Remove the DPBS and add Crystalline Trypsin solution (Cat. No. 03-047-1). The mixture should be stirred occasionally in the culture vessel at 37°C until detachment of cells.
5. After the cells have been removed from the growth surface, suspend them in diluted SBTI (Cat. No. 03-048-1) solution (0.1mg/ml) at a ratio of 5ml SBTI solution for each 1ml of Trypsin solution.
6. After separation of the cells from the microcarriers, resuspend the cells in NutriVero VP2 medium and transfer them in the desired concentration.

Cryopreservation of serum-free Cultures

1. Grow desired quantity of cells in T-flasks, harvesting when flasks are 80%- 100% confluent according to trypsinization procedure above.
2. After centrifuging, resuspend the cells pellet in cold serum-free freezing medium at a concentration of 3-5x106 cells/ml.
3. Freeze the cells gradually (1-2°C /minute) and store them in liquid nitrogen.
4. Viability and recovery of cryopreserved cells should be checked 24 hours after storage of vials in liquid nitrogen by following the thawing procedure outlined below.

Thawing of Cryopreserved cells

1. Thawing should be performed at 37°C.
2. Immediately after thawing, suspend the cells in serum-free growth medium at a ratio of at least 1:10.
3. After centrifuging, resuspend the cells at high concentration in growth medium.

Quality Control

NutriVero VP2 performance tested using Vero cells pre-adapted to serum-free culture in NutriVero VP2. Additional standard evaluations are pH, Osmolality and sterility tests.

Auxiliary products

Product	Cat. No.
Alanyl-glutamine solution	03-022-1
Crystalline Trypsin	03-047-1
Soybean Trypsin Inhibitor (SBTI)	03-048-1
Serum-Free Cell Freezing Medium	05-065-1

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