Plus™ VERO SFM

with L-glutamine without Sodium bicarbonate

Product Information Sheet

Introduction

Plus™ VERO Serum-Free medium（SFM）is a component-defined cell culture medium, formulated without any human or animal-derived components. Plus™ VERO SFM is designed to support the serum-free growth of VERO cell line of interest in the areas of virology, virus production, and biotechnology.

Caution: Not intended for human or animal diagnostic or direct therapeutic uses.

Storage Conditions: 2 to 8 °C, in the dark

Shelf Life: 1 year

Intended Use

Plus™ VERO SFM is a component-defined, animal component-free and ultra-low protein medium, designed specifically for the growth of VERO cell line. Plus™ VERO SFM is particularly suitable for growing viruses.

Features of Plus™ VERO SFM

- Very low protein concentration ~ 5 µg/mL
- No proteins or peptides of animal or human origin
- No complexes such as plant hydrolysate, yeast extract. Ease of downstream product purification
- Reduced risk of viral contamination
- Better Lot-to-lot consistency
- Equivalent cell growth and virus titers vs. serum-supplemented media

Preparing Liquid Medium

1. Measure out 5% less distilled water than desired final volume.
2. Add 14.98 g powder per liter to 15 to 30 °C (room temperature) D.I. water with gentle stirring.
3. Stir until dissolved. Please avoid extensive mixing with mixing time longer than 5 minutes.
4. Add 3.0 g of $NaHCO_3$ per liter of medium.
5. Stir until dissolved.
6. Adjust pH of medium according to Table 1: use of 1 N HCl is recommended.
7. Dilute to a desired volume with D.I. water.
8. Sterilize immediately by membrane filtration with low protein binding membrane. PVDF, or PES membrane is highly recommended.
9. Please store the culture medium in glass vessel, or plastic bag with LDPE contact layer at 2 – 8 °C. Plastic bag with EVA contact layer is not recommended.

Table 1.

Cell Culture Condition	pH Adjustment	Example
Culture with CO_2 buffering	0.2 – 0.3 below desired final working pH, e.g. 7.1 – 7.2	T-flasks, Roller bottles with vent
Culture without CO_2 buffering	0.5 – 0.7 below desired final working pH, e.g. 6.7 – 6.9	Roller bottles without vent

Cell Culture Procedures

Due to ultra-low protein concentration in Plus™ VERO SFM, care must be taken to protect cells from being damaged by digestion when making cell passage. We have found that modification on protocols may be required to obtain optimum results with Plus™ VERO SFM. The following protocols have been used successfully in our laboratories.

Adaptation of Cultures to Plus™ VERO SFM

For VERO cells apply to virus production or other application, if original cells are cultured in serum-contained culture medium, no adaptation is needed. If original cells are already adapted in other serum-free culture medium, or the directly culture in Plus™ VERO does not result a good cell growth, a weaning adaptation procedure might be required by mixing 50% original culture medium with Plus™ VERO and culture until sub-confluence. Around 3 to 4 passages might be required for the cells adapted to Plus™ VERO SFM.

Static Cultures

1. Remove media from T-75 cm^2 tissue culture flask (We recommend products from Corning Costar). Rinse flasks with 12 mL Dulbecco's PBS without Ca^{2+} or Mg^{2+} and remove.
2. Add 3 mL pre-warmed 0.05% Trypsin-EDTA to flask and dispense evenly over the entire surface area. Trypsin-EDTA is allowed to sit for 3 minutes at room temperature. Remove 2 mL Trypsin-EDTA and leave 1 mL on the T-flask.
3. Cultures are then incubated at 37 °C until cells become rounded (about 3 to 5 minutes depends on the confluency level) and are easily dislodged from the surface by shaking the flask. Avoid rapping the flask due to cells are more fragile in serum-free medium.
4. Once detached, add 12 mL Plus™ VERO SFM medium containing 500 ug/mL Soybean Trypsin Inhibitor (optional) to flask and transfer cell suspension to a 15 mL centrifuge tube.
5. Centrifuge for 5 minutes at 160 x g.
6. Remove supernatant and re-suspend cell pellet in 10 mL Plus™ VERO SFM medium.
7. Determine viable and total cell number. Determine cell viability by trypan blue dye exclusion method, and use a hemocytometer to determine cell number.
8. Seed flasks at $1.25\text{-}2.5\times10^4$ viable cells/cm^2.
9. Incubate at 37 °C in a humidified chamber containing of 5 to 8% CO_2 until cells are 80-90% confluent, which takes 3 to 4 days after seeding.

Roller Bottle Culture

Pre-warm 0.2-0.25 mL of medium per cm^2 of roller bottle surface area at 37 °C. Inoculate the roller bottle with about $1.25\text{-}2.5\times10^4$ cells/cm^2 to get an adequate distribution of cells in the bottle. Initial speed for the roller bottle should be 0.3-0.4 rpm. Cells will firmly attach within 12-24 hours. The speed of the roller bottle should be increased to 0.5-1 rpm after adequate inoculation.

Microcarrier Culture

Cell cultured in low protein media are more sensitive to mechanical stress. Conditions with higher shearing force may require modifications to the culture protocol with higher density of cell inoculums. In our laboratory, a 125 mL spinner flask was inoculated at $4x10^4$ cells/mg of beads with bead concentration of 5 mg/mL at recommended speed of 40-60 rpm. Total medium volume was 100 mL. We observed VERO cells attaching on Cytodex 1 microcarriers within 10 to 30 minutes after inoculation.

CelCradle™ Culture

Pre-warm 450 mL culture medium at 37 °C. Inoculate the bottle with about $1\text{-}1.5x10^8$ cells in a 50 mL culture medium and make the total culture medium volume 500 mL. The relative volume can be adjusted such as 480 mL culture medium and 20 mL culture medium with cells. Dispensing the cells on the top of matrix basket in order to achieve better distribution of cells on the carriers. Move the bottle on CelCradle Stage and start Run right after dispensing the cells. Set the parameter at Up: 2.0 mm/s, Down: 2.0 mm/s, T_H: 20 s, B_H: 0 s and let run for 3~4 hours. After seeding phase, switch the parameters to Up: 1.0 mm/s, Down: 1.0 mm/s, T_H: 10 s, B_H: 10 s for cell culture. Due to the medium contains more buffers for virus production, higher CO_2 concentration, e.g. 7% maybe required for the first day after inoculation if the culture medium tends to be with higher pH initially. Monitor the pH and residual glucose to decide if adjust of CO_2 or medium exchange is needed. Cells will reach confluence at about 5~6 days culture.

Quality Control Testing

The performance of Plus™ VERO SFM is tested for quality control using VERO cells. pH, osmolality of the medium are measured and tested for the absence of bacterial, fungal and endotoxin contaminants.

Contact Information

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