800-916-8311
www.InVitria.com
Info@InVitria.com

OptiVERO

Guidelines for Use

OptiVERO™
777OPT067-1L
Virus Production Media
Blood-free

Introduction

OptiVERO SFM supports the growth and expansion of VERO cells in T flasks and microcarriers. This medium contains only synthetic and recombinant components and does not include any animal or human serum-derived ingredients. When combined with an animal-free trypsin and trypsin inhibitor (not provided), this medium is blood free and chemically defined. OptiVERO SFM is designed for humidified incubators with 5% CO2 supplementation.

Long Term Storage

OptiVERO is supplied as a 50x Media Supplement, 100x Protein Supplement, and a 1x Base Media. The concentrated supplements should be stored at -20^{o}C in the dark. The Base Media should be stored at 4^{o}C. Separated, the media components are stable for up to 1 year. The complete media is stable for at least 6 months when stored at 4^{o}C. Do not freeze the complete medium.

Instructions for Use

Each 1000 mL of complete OptiVERO requires the addition of 20 mL of OptiVERO Media Supplement and 10 mL of OptiVERO Protein Supplement. This medium is not supplemented with antibiotics. If desired, add Gentamicin/Amphotericin (Life Technologies #R01510) at 0.1-0.5x final. Do not add to a 1x final. This medium contains HEPES and glutamine source. No further supplementation is needed.

To generate 1 liter of complete OptiVERO, thaw the OptiVERO Media Supplement and the OptiVERO Protein Supplement in a 37^{o}C water bath. Do not subject the Protein Supplement to multiple freeze thaw cycles. Once the supplements are completely thawed, mix the supplements by gently pipetting up and down. Do not vortex. Add 20 mL from the OptiVERO Media Supplement directly to the OptiVERO Base Media. Add 10 mL of OptiVERO Protein Supplement directly to the OptiVERO Base Media. Once antibiotics are added (optional), the media is ready to use. Avoid repetitive heating and cooling of the complete medium by withdrawing and prewarming only the volume needed in the final culture vessel to be used.

OptiVERO SFM contains minimal concentrations of proteins. Thus it is absolutely critical to neutralize and remove all traces of enzyme as excessive carry over will reduce OptiVERO SFM performance. Inclusion of soy bean trypsin inhibitor (not included) and/or high dilutions of enzyme with DPBS (i.e. 1-10) is highly recommended. See protocol for suggested amounts of enzyme and wash out volumes. 1x TrypLE + 1 mM EDTA was used during the development of this media (Life Technologies catalog number A1217701 in

addition to 0.25% Soybean Trypsin Inhibitor Life Technologies 17075-029). Soybean trypsin inhibitor should be reconstituted in DPBS and sterile filtered prior to use.

VERO Culture Adaptation from Serum

VERO cells can be seeded directly from serum-containing medium. Aliquot 0.2 mL/cm^2 OptiVERO SFM into the desired culture vessel and allow the media to equilibrate in the incubator for at least 30 minutes. Prewarm the desired enzyme of choice in a 37˚C water bath. This medium was developed using 1x TrypLE and 0.25% soy bean trypsin inhibitor. VERO cells should be washed with 0.5 mL/cm^2 DPBS twice to remove all traces of serum and removed from the growth surface using 0.05 mL/cm^2 TrypLE (NOTE: do not expose cells to the trypsin/TrypLE for longer than 6 minutes). Tap the side of the vessel to ensure all cells have been removed from the growth surface. After cell detachment is observed, add equal volume of 0.25% soy bean trypsin inhibitor followed by 0.2 mL/cm^2 DPBS to dilute out the enzyme. Collect the cells with 5-10 mL prewarmed media and pellet by centrifugation at 1000 RPM for 5 minutes. Cells should be resuspended in a minimal volume of prewarmed OptiVERO SFM and counted. Cells should be seeded at an initial cell density of 10,000-20,000 cells/cm^2. VERO cells should be subcultured every 3-5 days. When cells are ready for subculture, follow the procedure outlined above.

Microcarrier Culture

OptiVERO SFM was optimized specifically for the cultivation of VERO cells in microcarrier (MC) culture and can support high density culture of VERO cells for 8-10 days when combined with glucose and bicarbonate feed strategies (Figure 1). If longer runs are desired, please contact technical support to request a VERO-optimized amino acid feed.

MC Preparation

This medium was developed using Corning untreated plastic microcarriers (Ref 3772). Other surfaces have not been evaluated at this time. The typical surface area/mL for plastic microcarriers is shown in Table 1. Development runs consisted of 150 mL of media containing 10 cm^2/mL MCs for a total of 1500 cm^2 spinners.

Desired cm^2/mL	mg MC/mL media	Total mg MC/150 mL
10	28	4200
13	36	5400
15	42	6300

Table 1. Surface area to mass conversion for plastic MC

Vessel Preparation

Spinners should be washed and dried according to in house procedures and subsequently autoclaved to sterilize. Allow the spinners to cool for at least 4 hours prior to starting the inoculation process. When the MCs are washed and ready, add 100 mL of cold OptiVERO media to the spinner immediately followed by

800-916-8311
www.InVitria.com
Info@InVitria.com

the addition of the MCs to the vessel. Do not allow OptiVERO SFM to warm in the spinner without microcarriers as excessive adsorption of media components to the glass will affect cell adhesion to the MCs. NOTE: MCs add ~7-20 mL of volume depending on the amount of MCs and residual volume from the MC washes. Therefore, adjust the amount of media used to transfer the MCs into the vessel. Total volume of spinner after MC addition should be approximately 135 mL. The tube containing the MCs should be washed with an additional volume of OptiVERO SFM to be sure to transfer all of the MCs. Transfer the spinner to the incubator and begin stirring the MCs at 40-50 RPM. Allow the spinner to equilibrate for at least 2 hours prior to the inoculation of the VERO cells.

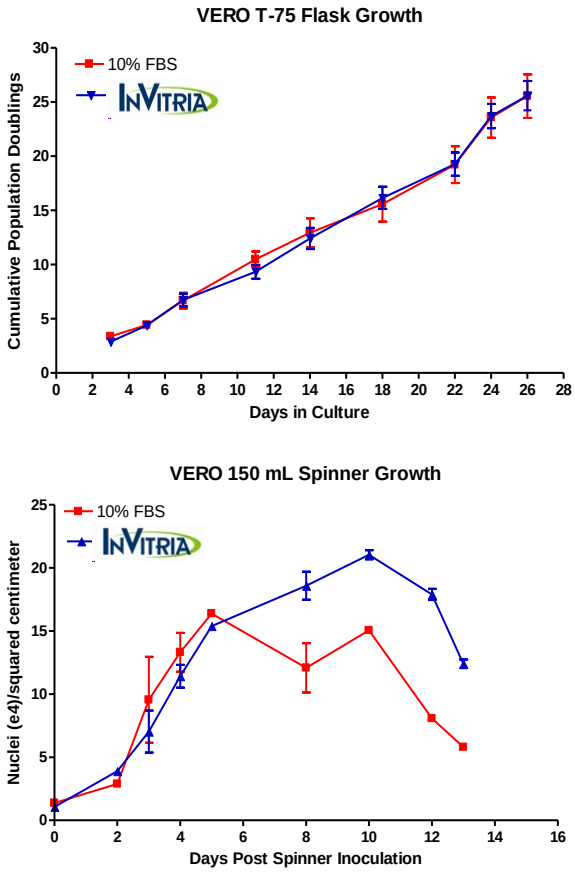

Figure 1. VERO Cell growth in T-75 Flasks and 150 mL Spinners.

Cell Inoculum Preparation

VERO cells at 70-80% confluence in 2 T-150s is generally adequate to seed 1 spinner at an initial cell density of 10,000 cells/cm^2. Cells should be washed and trypsinized according the subculture section of this protocol. Cells should be pelleted and resuspended in 5 mL of prewarmed OptiVERO SFM. Prior to seeding cells into the spinner, it is crucial to pass the cell suspension through a 0.7 µm nylon cell strainer (Corning 431751 or equivalent) in order to ensure a single cell suspension. Cells should be counted and the cell suspension should be adjusted to 1e6 viable cells/mL in a total of 15 mL of OptiVERO SFM. Obtain the equilibrated spinner. While the spinner is stirring at 40 RPM in the hood, slowly add the 15 mL of 10x cell suspension. Place the spinner back in the incubator and do not disturb the spinner for at least 4 hrs.

Performing an MC run

Starting at Day 2, spinners should be monitored every day for culture glucose levels and pH. Culture glucose should be maintained at 2 g/L final by the addition of a 45% glucose solution. Culture pH should be maintained between 6.9 and 7.2 via the addition of 7.5% Sodium Bicarbonate solution. If desired, culture samples can be obtained at this time to monitor cell growth and quantified by method of choice. Runs with OptiVERO in comparison to 10% FBS and a popular serum free media are shown in Figure 1.