OptiVERO - Virus Production Using VERO Cells in Flasks without Serum in Chemically Defined Cell Culture Media

Application Note

Workflow Summary

Combine kit supplements of OptiVERO. Prepare complementary reagents. Wash cells to remove traces of FBS and harvest from growth surface. Seed in OptiVERO and grow to confluence. Add desired virus to be expanded in minimal basal EMEM. Incubate for 2 hours at 37°C. Add fresh OptiVERO. Allow virus expansion for 3-13 days. Quantify virus by desired method.

Introduction

VERO cells are one of the most common continuous cell lines used in laboratory and industrial settings. While they have many important applications in research, one of the most critical is as a preferred cellular substrate for virus production [1]. VERO cells are traditionally grown in 10% fetal bovine serum (FBS) and exhibit commercially acceptable doubling times and viability. However, current guidance by regulatory bodies in addition to Good Manufacturing Practice (GMP) compliant manufacturing of cell-based, advanced therapy medicinal products (ATMPs) states that alternatives to FBS should be utilized due to poor reproducibility and safety concerns associated with FBS use [2,3]. Popular serum free media (SFM), such as VP-SFM, contain high levels of hydrolysates that can cause batch-to-batch variability. OptiVERO was designed to eliminate these concerns and is a chemically defined and blood-free (serum free) media. Here, we compare VERO cell growth and virus production in OptiVERO vs FBS containing media in 2D cell culture flasks.

Materials and Equipment

Growth Media Preparation

- OptiVERO Components: Base Media, 50X Protein Supplement
- Gentamicin/amphotericin (Life Technologies R01510)
- L-Glutamine

Cell Passage

- 75 cm^2 tissue culture flask (Falcon 353136)
- 150 cm^2 tissue culture flask (Falcon 355001)
- Soybean trypsin inhibitor (Life Technologies 17075-029)
- TrypLE (Life Technologies A1217701)
- 100 mM EDTA (Sigma Aldrich E6758)
- Dulbecco's phosphate buffered solution (VWR VWRL0119-1000)
- 50 mL conical tubes (VWR 76211-286)

Equipment

- 5% CO2 incubator at 37¬∞C, saturating relative humidity
- Class A2 biological safety counter
- Centrifuge
- Cell counter or hemocytometer

Optional

- VP-SFM (Thermo Fisher 11681020)
- Minimum essential medium (MEM) with Earle's salts
- L-glutamine (Corning 10-010-CV)
- Fetal Bovine Serum (FBS)

Protocol

Growth Media Preparation

1. To generate 1 liter of complete OptiVERO, thaw one OptiVERO Protein Supplement (20 mL) in a 37°C water bath. Do not subject the Protein Supplement to multiple freeze-thaw cycles.
2. Once the supplement is completely thawed, gently stir each supplement by pipetting up and down. Do not vortex.
3. Add 20 mL from the OptiVERO Protein Supplement directly to the OptiVERO Base.
4. Add 10 mL of OptiVERO Protein Supplement directly to the OptiVERO Base Media.
    a. This medium requires the addition of L-Glutamine to a final concentration of 4 mM. If desired, add gentamicin/amphotericin to 0.1-0.5x final. Do not add to a 1x final concentration.
    b. Avoid repetitive heating and cooling of the complete medium by withdrawing and pre-warming only the volume needed in the final culture vessel to be used.

Stock-Reagent Preparation

50x Soybean trypsin inhibitor

1. To a 1 g container of soybean trypsin inhibitor, add 8 mL of DPBS and allow the powder to reconstitute at room temperature for at least 3 hours prior to use. Avoid the excessive formation of bubbles during reconstitution by allowing the vial to sit undisturbed.
2. Upon successful reconstitution, pipette the solution up and down with a 10 mL pipette to achieve adequate mixing.
3. Pass the solution through a 0.22 µm PES syringe filter into a sterile 15 mL tube.
4. Aliquot solution into (12) 650 µL aliquots in 1.5 sterile tubes and store at -20°

100mM EDTA

1. Weigh 1.86 g of dry EDTA on an analytical balance and bring the solution up to 50 mL with DPBS.
2. Once the EDTA has dissolved into solution, sterile-filter the solution using a 0.22 µm PES syringe filter into a sterile 50 mL tube.

3. Store the solution at 4°C for up to 6 months.

1x TrypLE + 1 mM EDTA

1. To a sterile 250 mL media bottle, aseptically add 222.5 mL of DPBS followed by 25 mL of 10x TrypLE and 2.5 mL of 100 mM EDTA.
2. Store the solution at 4°C for up to 6 months. This solution may be heated and cooled repeatedly without affecting the activity of the enzyme.

Working Reagent Preparation

1. Dilute 50X soybean trypsin inhibitor to 1x in dPBS (0.05 mL/cm^2 volume needed).

Culture Adaptation to Serum-Free Medium

For additional information about VERO cell growth and maintenance in OptiVERO, please refer to the Application Note: ***"Expansion of VERO Cells Without Serum in Chemically Defined Cell Culture Media"***

1. Aliquot 0.2 mL/cm^2 OptiVERO into the desired culture vessel and allow the media to equilibrate in the incubator for at least 30 minutes. Prewarm the diluted soybean trypsin inhibitor and 1x TrypLE in a 37°C water bath.
2. Wash VERO cells with 0.5 mL/cm^2 dPBS twice to remove all traces of serum.
3. Remove cells from the growth surface using 0.05 mL/cm^2 TrypLE/EDTA (NOTE: do not expose cells to the TrypLE/EDTA for longer than 6 minutes)
4. Tap the side of the vessel to ensure all cells have been removed from the growth surface.
5. After cell detachment is observed, add an equal volume of soybean trypsin inhibitor followed by 0.2 mL/cm^2 DPBS to dilute out the enzyme. Collect the cells in a 50mL conical tube.
6. Wash the surface and collect any remaining cells with 5-10 mL prewarmed media.
7. Pellet by centrifugation at 1000 RPM for 5 minutes.
8. Resuspend cells in a minimal volume of prewarmed OptiVERO and count.
9. Seed cells at an initial cell density of 10,000-20,000 cells/cm^2. VERO cells should be sub-cultured every 3 to 5 days.
10. At time of subculture, repeat the procedure above.

Virus Production in Tissue Culture Flasks

1. Prior to infection studies, VERO cells should be allowed to adapt to OptiVERO for at least 3 passages. VERO cells should be grown to confluence in the desired flatware.
2. For viral inoculation, 75% of the growth media should first be removed
3. The virus to be expanded should be inoculated at 0.001 - 0.01 PFU/cell in basal (0.06 mL/cm^2).
4. To ensure uniform infection over the entire monolayer, VERO cells should be incubated at 37°C for 90 minutes after inoculation, with gentle rocking of the flask every 15 minutes.
5. Add 0.7mL/cm^2 of fresh, prewarmed OptiVERO to each flask
6. Specific protocols for virus propagation are dependent on virus type. For the attenuated Dengue strain used here, for example, culture glucose (2 g/L) and neutral pH were maintained by the addition of 45% glucose and 7.5% sodium bicarbonate solutions, respectively.
7. Samples should be removed daily for virus quantification

Results and Discussion

Here, a blood- and serum-free, chemically defined virus-production media known as OptiVERO is evaluated for its ability to support VERO virus production, compared to media supplemented with FBS and to VP-SFM.

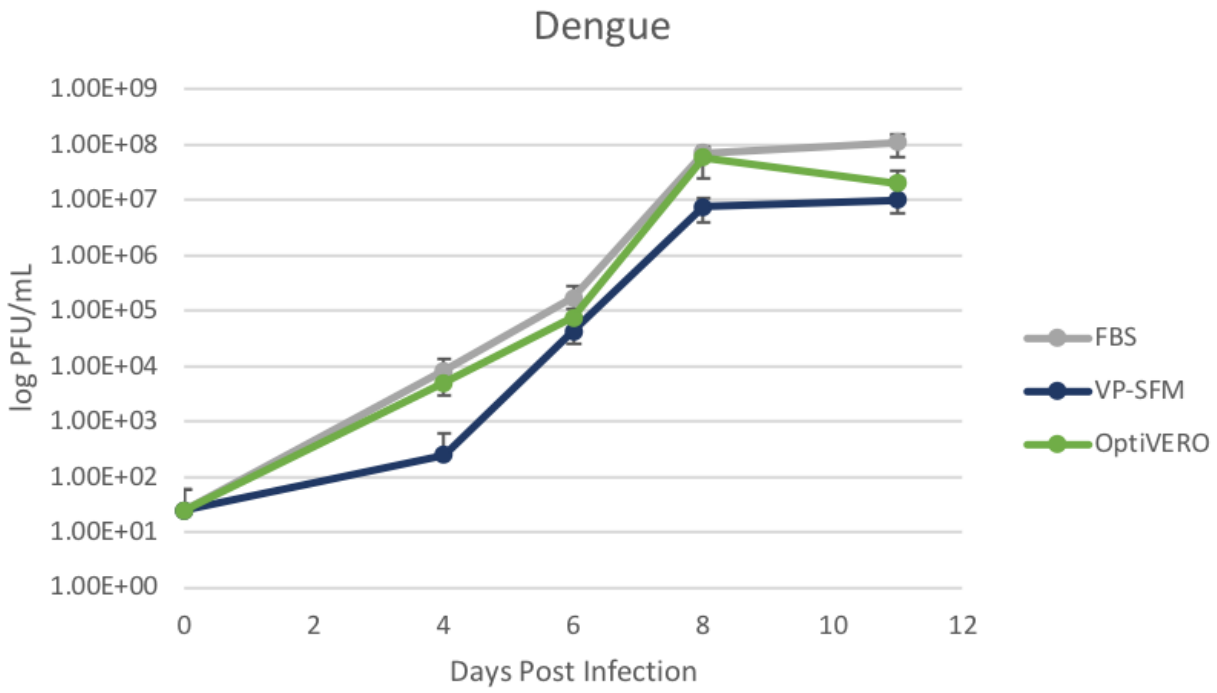

Figure 1 Dengue virus production in Vero cells cultured in 10% FBS, OptiVERO, and VP-SFM

An attenuated Dengue strain was used as a model virus to demonstrate the virus production capabilities of each medium (Figure 1). Dengue was propagated for 12 days, with samples removed for plaque titration every other day. The Dengue virus growth plateaued at day8 and was determined to be 7.25E+7, 7.5E+6, 6.00E+7 PFU/mLfor EMEM + 10% FBS, VP-SFM + 4 mM Glutamine, and OptiVERO, respectively. These data show that VERO cells grown in OptiVERO virus production capabilities similar to those grown in 10% FBS and exhibited superior virus production to hydrolysate-containing medium, VP-SFM.

References

1. Ammerman NC, Beier-Sexton M, Azad AF. Growth and maintenance of Vero cell lines. Curr Protoc Microbiol 2008;Appendix 4:Appendix 4E.
2. Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3). Official Journal of the European Union 2011;C73:1-18.
3. van der Valk J, Bieback K, Buta C et al. Fetal Bovine Serum (FBS): Past - Present - Future. ALTEX 2018;35:99-118.

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