Vaccine manufacturing

OptiPRO SFM AGT Medium supports viral vaccine production scale-up with consistent and equivalent performance compared to liquid format

Introduction

Gibco™ OptiPRO™ SFM is a well-established medium in the viral vaccine industry, serving approximately 2,000 customers. It is recognized for delivering strong cell growth and viral titer production with adherent kidney-derived cells. The formulation is known for its serum-free, animal origin–free (AOF) status and very low protein levels (<10 µg/mL). OptiPRO SFM has been available in a liquid format for many years and has recently been made available in the Gibco™ Advanced Granulation Technology™ (AGT™) format to support larger-scale viral vaccine production. Selecting a medium early in development that can also meet future production needs is essential for allowing streamlined production scale-up.

Liquid media are often ideal earlier in development at smaller scales, as they are ready to use and require few additional preparation steps. However, at larger scales later in development, a dry medium format is typically considered more cost-effective as it can reduce shipping and storage footprint requirements. The finely ground dry powder medium (DPM) format has been the traditional option. However, another well-established option is the AGT format, which is manufactured through a technologically advanced process that enables the production of a complete media formulation in a granular form. A medium in AGT format can offer several benefits over the traditional DPM:

- Faster dissolution
- Lower dust generation
- A simplified, more rapid medium preparation workflow
- Up to 50% reduction in labor time and costs [1]

The AGT granular form provides a greater surface area for faster particle dissolution and lower dust generation. In addition, an AGT medium is a complete pH and osmolality preadjusted formulation. These features can simplify and reduce the number of steps in a medium preparation workflow. Users have reported that an AGT medium can provide up to a 50% reduction in labor time and costs over using a DPM [1]. Additionally, these AGT features may also provide less risk associated with variation and errors in media preparation.

To enable a streamlined format transition at scale-up, the dry medium format must perform in a consistently comparable manner to the liquid. Therefore, a study was conducted to evaluate the growth and productivity performance of Gibco™ OptiPRO™ SFM AGT™ Medium compared to the liquid format. Two lots of each media format were tested with adherent Vero cells for growth over four passages and vesicular stomatitis virus (VSV) production. The media performance was evaluated based on assessments of viable cell density (VCD), cumulative population doublings (CPD), and VSV titer production by plaque assay.

Materials and methods

Reconstitution of AGT medium

1. The vessel was filled with room temperature (15°C to 30°C) water for injection (WFI)-quality water to 90% of the final volume and agitation was started.
2. OptiPRO SFM AGT Medium was gradually added and mixed for 30 minutes after the final addition.
3. WFI was added at a quantity sufficient (QS) to reach final volume and mixed for 10 minutes.
4. The pH and osmolality were measured and recorded.
5. The medium was sterile filtered by positive pressure membrane filtration.

Cell culture

Vero cells banked in OptiPRO SFM liquid format were recovered for two passages in T-75 flasks in OptiPRO SFM liquid format. After recovery, cells were transferred and passaged four times in duplicate T-75 flasks with two different lots each of OptiPRO SFM in liquid and reconstituted AGT formats, each with 4 mM Gibco™ GlutaMAX™ Supplement. Cells were cultured at 37°C with 5% CO_2 in 20 mL of medium with passaging every 3 to 4 days at a seeding density of 3 x 10^4 cells/cm^2 for a 3-day culture and at a seeding density of 2 x 10^4 cells/cm^2 for a 4-day culture. VCD and viability were measured using a Vi-CELL™ BLU analyzer (Beckman Coulter).

Infection and harvest

Cells were infected with VSV on day 4 after the fourth passage in each test medium at a multiplicity of infection (MOI) of 0.0001 in triplicate T-75 flasks. Total culture was harvested approximately 3 days postinfection when a cytopathic effect (CPE) of ≥80% was observed. At harvest, the supernatant was collected for viral quantification after one freeze-thaw.

Virus quantification by plaque assay

Vero cells were seeded in a 24-well plate. Once the cell monolayer was confluent, cells were washed and infected with 10-fold diluted viruses. After incubation, the plates were fixed and stained, and the plaques in each well were counted manually.

Results

The reconstitutions of two lots of OptiPRO SFM AGT Medium showed that a total mixing time of 40 minutes was adequate to solubilize each lot of the single-part complete medium formulation without any adjustments to pH or osmolality. The pH and osmolality results of the reconstituted AGT medium were within the acceptable product specification ranges. In addition, the pH and osmolality results were shown to be comparable to the catalog liquid lots with no calculated difference in pH and a 2.8% difference in osmolality between the formats (Table 1).

Table 1. Test medium pH and osmolality results.

OptiPRO SFM	Lot number (last 4 digits)	pH	Osmolality (mOsm/kg)
Liquid format	9108	7.3	301
Liquid format	9869	7.2	303
Average, liquid format		**7.3**	**302**
AGT format	3021	7.3	312
AGT format	2066	7.2	309
Average, AGT format		**7.3**	**311**
Difference of AGT format from liquid format (%)		**0.0%**	**2.8%**

Across four passages, Vero cell growth with the OptiPRO SFM AGT format supported average cumulative population doublings comparable to those with cell growth with media in the liquid format; average cumulative population doublings were also comparable between lots of media in both formats (Figure 1). In addition, Vero cells grown with the OptiPRO SFM AGT format exhibited similar VCD to cells grown with the media in liquid format across four passages (Figure 2). Cell viability remained comparably high across all four passages at an average of 96% to 99% for both liquid and AGT media formats and all lots tested (data not shown). Based on plaque assay results, VSV average titer productivity was also shown to be comparable between the AGT and liquid media formats (Figure 3).

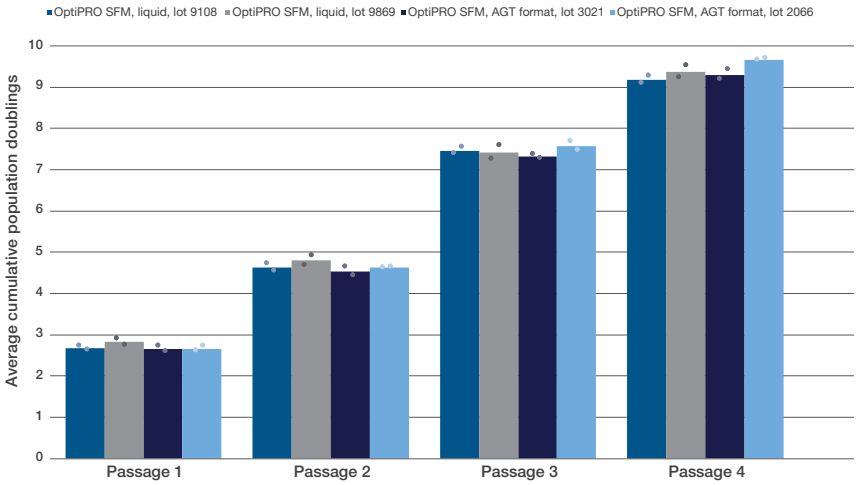

Figure 1. Cumulative population doublings. Across all four passages, the Vero cell average cumulative population doublings from cells grown in OptiPRO SFM liquid format and those from cells grown in OptiPRO SFM AGT format were shown to correspond highly; additionally there was high correspondence between lots of both formats. Dots indicate results for each flask (n = 2).

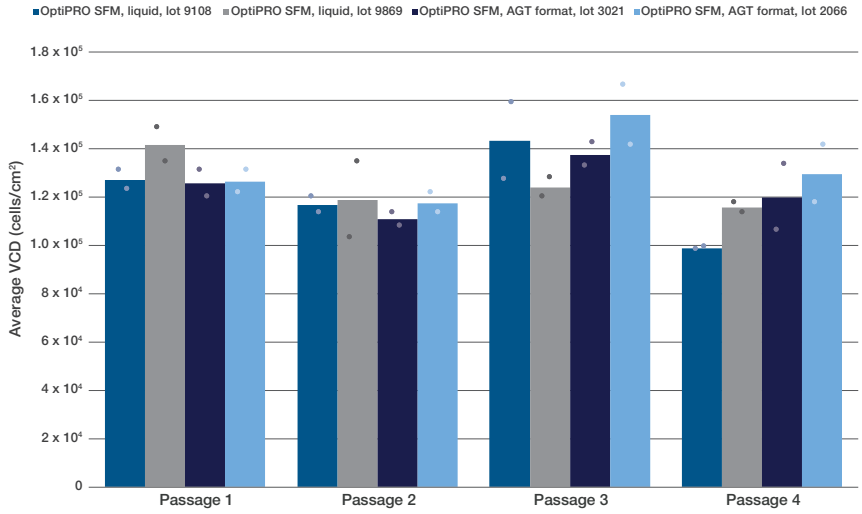

Figure 2. VCD. Similar average VCD of Vero cells was observed across the four passages with the OptiPRO SFM AGT format and the OptiPRO SFM liquid format. Dots indicate results for each flask (n = 2).

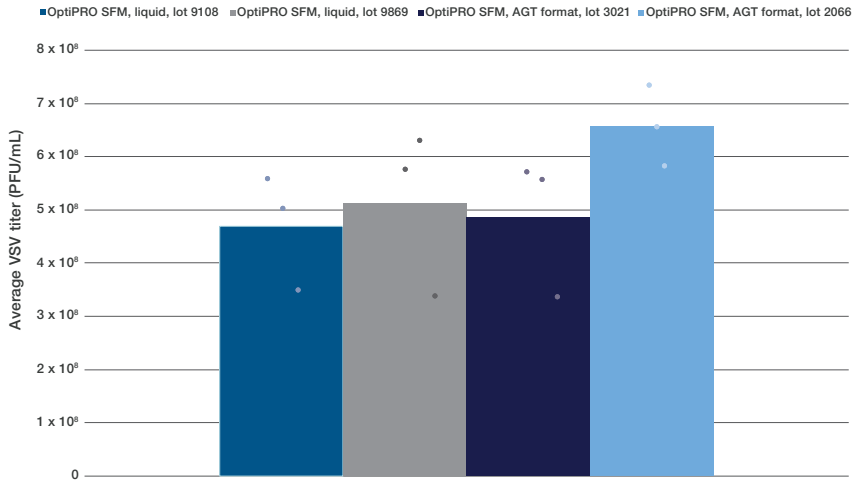

Figure 3. VSV production. Based on plaque assay results, Vero cells grown with the OptiPRO SFM AGT format produced a relative average VSV titer comparable to that from cells grown with the liquid format. (Virus quantifications were based on average plate counts of two sample dilutions.) Dots indicate results for each 24-well plate (n = 3).

Conclusions

In combination, OptiPRO SFM liquid and OptiPRO SFM AGT formats can support consistent, streamlined viral vaccine production from development to clinical trials and commercial production. The liquid format is optimal for small-scale development, and the AGT format is favorable for larger-scale production. The AGT format provides a complete pH and osmolality preadjusted medium that enables simple reconstitution in as little as 40 minutes. The study results outlined here confirmed the OptiPRO SFM liquid and AGT formats support comparable and consistent cell growth and viral productivity.

In addition, our global manufacturing programs are designed for equivalency across locations, helping to deliver reliable supply assurance with consistent product quality. Lastly, our field application specialists and R&D scientists can be available for consultation and guidance on the issues or questions you may need to address. Together, the features and performance of OptiPRO SFM and OptiPRO SFM AGT Medium, along with reliable supply availability and technical expertise, can help enable the accelerated development and streamlined scale-up of your viral vaccine production process.

OptiPRO SFM and OptiPRO SFM AGT Medium

- Serum-free, AOF, and very low protein (<10 µg/mL) formulation
- Liquid and dry AGT formats with demonstrated consistent and comparable performance
- Liquid format offered in 100 mL and 1,000 mL bottles
- AGT format offered in 10 L and 100 L equivalent containers

Reference

1. Deets Beat, Inc. (2014) Advanced Granulation Technology™ (AGT™ dry media format) **Culture Media: Benefits and Case Studies**.

Ordering information

Description	Quantity	Cat. No.
OptiPRO SFM	100 mL	12309050
	1,000 mL	12309019
OptiPRO SFM AGT Medium	10 L	A4000164001
	100 L	A4000164002
GlutaMAX Supplement	100 mL	35050061
	20 x 100 mL	35050079

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