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Application Note

Optimized Cell Culture Medium for Scalable Viral Vector Production

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Since the first FDA approval of a gene therapy product in 2017 (Kymriah®),1 the industry has continued to tap into the potential of cell and gene therapies (CGTs), resulting in a deepening pipeline ripe with opportunity. Viral vectors facilitate delivery of genomic material into cells, offering a wide range of advantages over non-viral gene delivery, such as low immunogenicity and high *in vivo* expression levels. The most used viral vectors, adeno-associated virus (AAV) and lentivirus, are traditionally produced in adherent cell culture using generic, unoptimized media supplemented with fetal bovine serum. Among other challenges, this can limit cell growth and overall viral vector productivity and introduce potential contaminants into the manufacturing process.

To meet the ever-increasing demand for CGTs, the industry must shift away from this inefficient and risky approach and adopt fit-for-purpose tools that enhance upstream operations. Our new cell culture medium offers an improved alternative to classical formulations that can help address the constraints in viral vector manufacturing and scale-up by breaking through the performance ceiling threatening the future of this exciting field of medicine.

The Limitations of Adherent Cell Culture

Bringing a CGT to market is a costly and complex process that requires specialized expertise and capabilities. Drug manufacturers that want to take advantage of this growing area of the industry—and CDMOs that want to partner with them—need to ensure they have the fastest route to commercialization, especially when it comes to viral vector production. Many of the legacy processes and technologies can efficiently produce enough viral vectors for the smaller batch sizes needed in the early days of gene therapy. However, these methods fall short in meeting the process economics and scalability demands of viral vectors when expanding to larger patient populations. Just as the industry rallied to improve productivity for monoclonal antibodies (mAbs) through new, innovative tools and techniques, the same needs to be done for CGTs and their key manufacturing component, viral vectors.

One area of focus is adherent cell culture, where cells grow by attaching to a modified plastic surface, such as in T-flasks or multilayer flasks. Adherent cell growth is limited by the surface area of the culture vessel, so scale-out requires operators to detach cells from the growth surface during passaging, requiring multiple rounds of manipulation in a biosafety cabinet. Batch size is limited to the maximum size of commercially available adherent culture vessels, combined with the time needed for an operator to handle a single vessel. Overall, this is a labor-intensive task that increases the risk of contamination because of the repeated open manipulation in biosafety cabinets, and it quickly reaches a practical batch size limit as demand increases.

Traditional methods also rely on undefined media, including components such as hydrolysates and/or sera, that contain growth factors and other nutrients to stimulate cell growth. The most frequently used serum, fetal bovine serum, provides a robust culture system but, due to its animal origin, introduces adventitious agent risk into the process and the final product. As the CGT market evolves, regulatory agencies are focused on adapting their requirements in tandem with scientific and clinical breakthroughs, resulting in increased stringency in expectations and processes. Eventually, it may become nearly impossible to support adherent processes due to the risk of animal-containing media. This is reminiscent of the mAb evolution when CHO cells moved to the forefront of recombinant protein production only after being grown in suspension using animal origin-free media.

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With the challenges of viral vector manufacturing in mind, we developed our EX-CELL® CD HEK293 Viral Vector Medium to provide an easier, faster, and more economical option for cellular growth that can efficiently move a gene therapy from bench to patient.

Improved Cell Growth and Viral Productivity with EX-CELL® CD HEK293 Viral Vector Medium

The EX-CELL® CD HEK293 Viral Vector Medium is a chemically defined, animal component-free cell culture medium optimized for transient transfection and virus production. The formulation is designed to support suspension cell culture rather than the traditional adherent process. This allows scaleup to much larger volumes utilizing bioreactors currently available on the market. Single-use bioreactors also introduce two key advantages: first, they allow for continuous monitoring and control of the culture system's physical and chemical parameters, such as temperature and pH; second, they reduce risk and validation time in using pre-sterilized, ready-to-use consumables. Bioreactor culture also paves the way for process intensification, as parameters for the growth, transfection, and production can be tweaked to make for a more effective process. Initial titers can also be higher with a suspension process, leading to increased output and lower manufacturing costs.

EX-CELL® CD HEK293 Viral Vector Medium is formulated to support high suspension cell densities with minimal clumping; high transient transfection efficiency when used with polyethyleneimine (PEI) as the transfection reagent; and high viral titers in shake flask and stirred tank bioreactor formats. The medium also supports lipid-based transfection protocols in addition to PEI. This medium does not require a post-transfection exchange or feed when used in a batch production process. It is part of the VirusExpress® Lentiviral Production Platform and the medium of choice for VirusExpress® 293T Lentiviral Production Cells. In addition, it is suitable as a stand-alone solution for other HEK293 and HEK 293T cell lines used for AAV, lentivirus, and adenovirus manufacturing.

As an animal component-free media, EX-CELL® CD HEK293 Viral Vector Medium supports cell growth and viral productivity without the use of any raw materials or supplements of animal or human origin. Using a chemically defined medium not only improves safety but also eliminates the performance variability commonly observed in animal-derived supplements, driving consistency from batch to batch. It also simplifies downstream purification and reduces risk in the product supply chain. As a product moves through clinical trials and toward commercialization, establishing a robust, regulatory-friendly cell culture process in the early stages of development mitigates the need for changes to accommodate increasing scale or quality requirements. This can help avoid delays that come from reworking the process and addresses potential concerns from regulatory authorities.

Comparing EX-CELL® CD HEK293 Viral Vector Medium To Legacy And Competitor Formulations

To illustrate the improved cell growth and viral productivity with EX-CELL® CD HEK293 Viral Vector Medium, its performance was evaluated against our legacy and competitor formulations. Although the legacy formulation met original design specifications, we recognized the opportunity for continuous improvement, hence the development of EX-CELL® CD HEK293 Viral Vector Medium.

Figure 1 shows a comparison between the growth profile of EX-CELL® CD HEK293 Viral Vector Medium and our legacy prototype formulation (CD Prototype I) in a Mobius® 3 L single-use bioreactor. The CD Prototype I values are based on an average of three separate runs. **Figure 1** shows a comparison between the growth profile of EX-CELL® CD HEK293 Viral Vector Medium and our CD Prototype 1 in a Mobius® 3 L single-use bioreactor using a third-generation packaging system with a GFP+pac transgene. The results indicate improved overall growth of cells when using the EX-CELL® CD HEK293 Viral Vector Medium. The improvement in titer is shown in **Figure 2**.

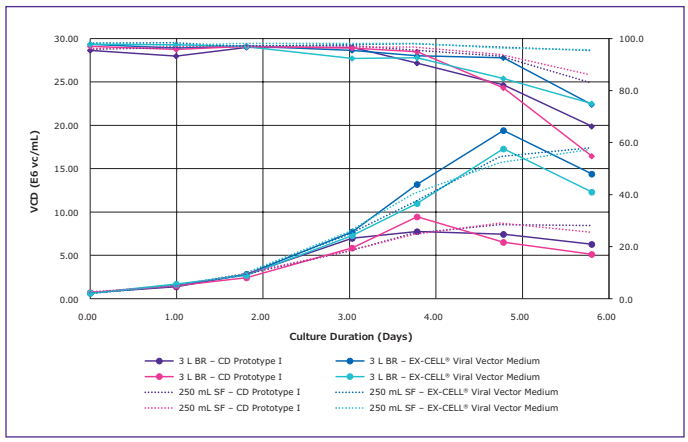

Figure 1. VirusExpress® 293T Lentivirus Cells grown in the Mobius® 3 L single-use bioreactor

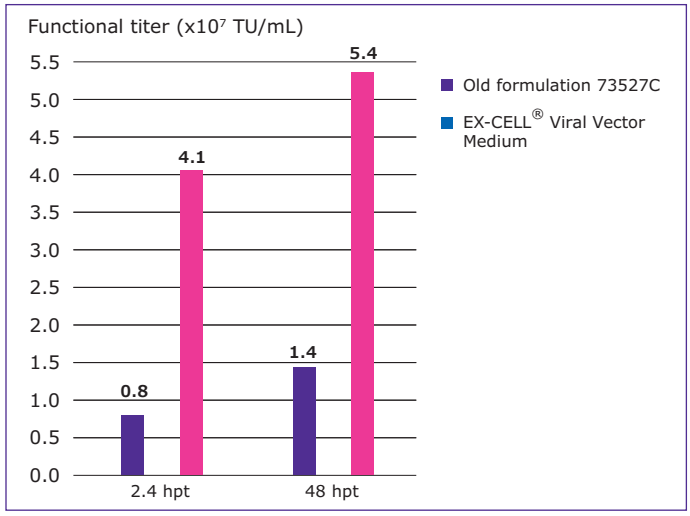

Figure 2. VirusExpress® 293T lentiviral productivity in Mobius® 3 L single-use bioreactor

Next, the performance of EX-CELL® CD HEK293 Viral Vector Medium in a clinical-scale bioreactor (50 L) was compared to that of the CD Prototype 1. **Figure 3** shows consistent results at this larger scale.

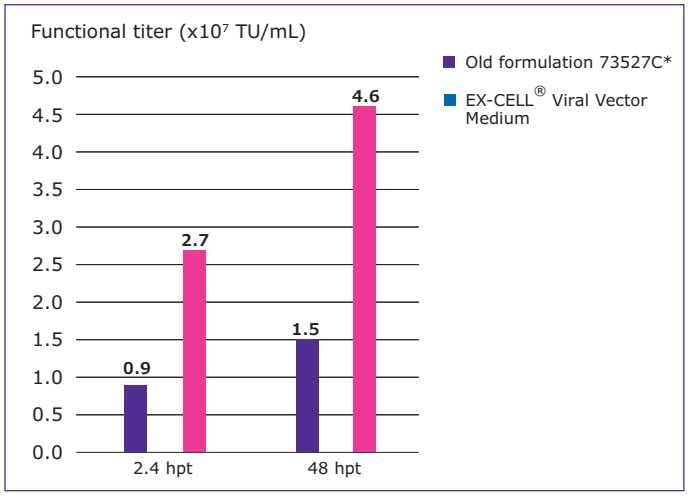

Figure 3. VirusExpress® 293T lentiviral productivity in Mobius® 50 L single-use bioreactor

In a study of direct competitor media, a considerably higher cell density in shake flasks was obtained when using the EX-CELL® CD HEK293 Viral Vector Medium (**Figure 4**). Competitors A and C required supplementation with an anti-clumping reagent to maintain a suspension culture with few to no cell clumps.

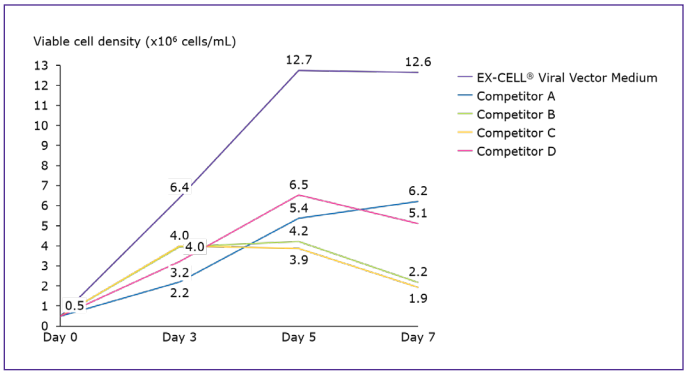

Figure 4. VirusExpress® 293T cell growth assay in shake flasks

Finally, viable cell density at time of transfection and at 48 hours after transfection when cells were grown in competitor medium in shake flasks is shown in **Figure 5**. Corresponding lentiviral productivity at 48 hours after transfection in shake flasks is shown in **Figure 6**. These studies demonstrate that EX-CELL® CD HEK 293 Viral Vector Medium significantly outperforms the competitor formulations in the functional titer produced by VirusExpress® 293T Lentiviral Production Cells.

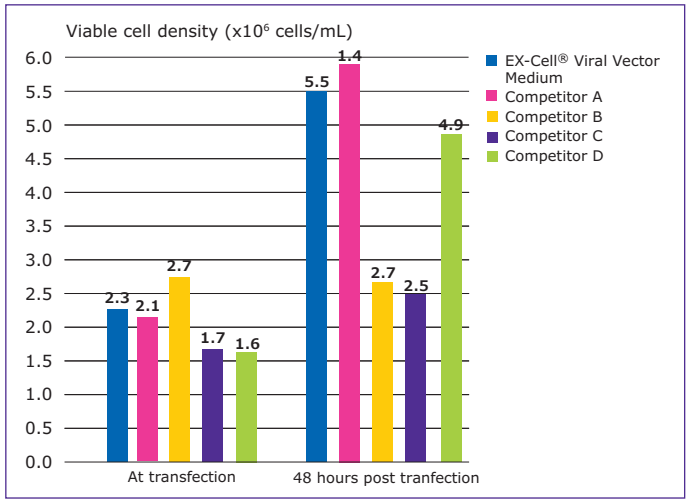

Figure 5. VirusExpress® 293T transfection viable cell density in shake flasks

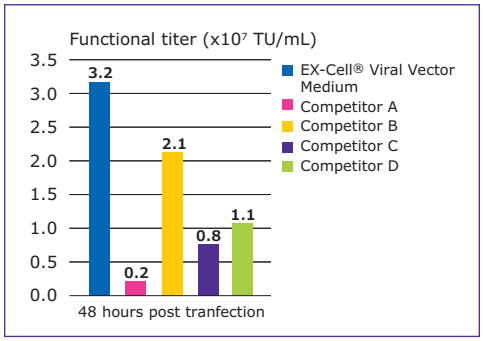

Figure 6. VirusExpress® 293T lentiviral productivity in shake flasks

Although a cell culture media's performance is critical for viral vector production, the reliability of the company that supplies it is equally important. Partnering with a manufacturing leader that can reliably supply media at the scales, in the formats, and within the timelines needed ensures the ability to consistently manufacture product to meet patient need now and well into the future.

The improved performance of EX-CELL® CD HEK293 Viral Vector Medium — combined with our extensive experience and expertise in media manufacturing and vector production — gives CGT manufacturers the tools they need to be successful as they explore the possibilities of one of the fastest-growing segments in today's regenerative medicine market.[2]

1. FDA. (August 2018). Remarks on FDA approval of first gene therapy in the United States. https://www.fda.gov/news-events/speeches-fda-officials/remarks-fda-approval-first-gene-therapy-united-states-08302018
2. Global News Wire. (August 2020). The global cell and gene therapy market by revenue is expected to grow at a CAGR of over 30.90% during the period 2019–2025. http://www.globenewswire.com/news-release/2020/08/04/2072578/0/en/The-global-cell-and-gene-therapy-market-by-revenue-is-expected-to-grow-at-a-CAGR-of-over-30-90-during-the-period-2019-2025.html

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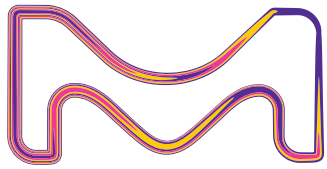

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