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# Evaluating the impact of flexible environmental controls on HEK293 cell growth and viability


## Abstract

This study evaluates the role of the INFORS HT Multitron incubator shaker in improving HEK293 suspension cell cultures for gene therapy applications. Key parameters such as viable cell density, viability, and glucose consumption were monitored under variable conditions to assess how the Multitron incubator shaker supports scalable bioprocesses. The results underline the Multitron's flexibility in providing an ideal environment for maintaining high viability and optimal cell density, making it highly suitable for gene therapy-related applications, including viral vector production.



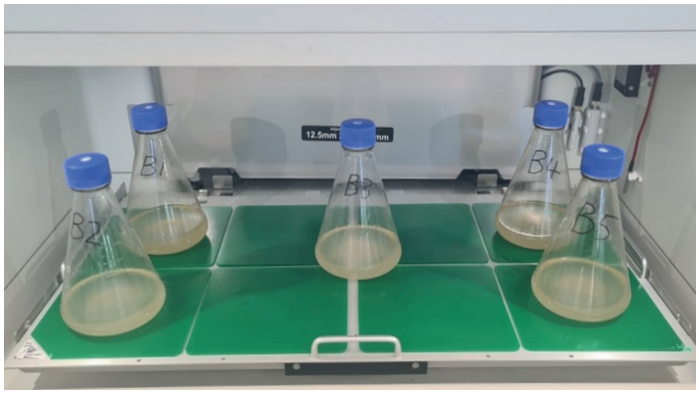
**Keywords**
HEK293 cells, suspension culture, incubator shaker, Multitron, orbit diameter, cell viability, cell density


## Introduction

HEK293 cells are widely used in gene therapy, particularly in the production of viral vectors, such as adenoviruses and lentiviruses, which are used for delivering therapeutic genes. These cells are prized for their high transfection efficiency, rapid growth rates, and ability to support the replication of various viral vectors. As the demand for gene therapy continues to grow, improving the culture conditions for HEK293 cells is essential to improve productivity, ensure scalability, and maintain the quality of viral vectors used in clinical applications.

To address these challenges, the INFORS HT Multitron incubator shaker offers parameter flexibility and precise environment control that can be tailored to the specific needs of HEK293 cell culture for gene therapy. By adjusting key parameters such as temperature, $CO_2$ concentration, agitation, relative humidity, and orbit diameter, researchers can create optimal conditions that maximize cell growth and viability.

This study evaluates how the Multitron incubator shaker's ability to adjust these parameters enhances the culture of HEK293 cells, supporting the efficient production of viral vectors.




**Flasks during the experiment inside the Multitron incubator shaker**

In collaboration with the Jefferson Institute for Bioprocessing (JIB), this research demonstrates how controlled environmental conditions can drive high cell densities and improve productivity of HEK293 cell-based bioprocesses.

## Objective

- Evaluate the performance of the Multitron Incubator shaker for improving HEK293 cell cultures in suspension for gene therapy.
- Assess the impact of different orbit diameters on cell growth, viability, and metabolic activity, relevant to viral vector production.

## Methods and materials

- **Media:** Gibco™ Freestyle 293 expression media with supplements including 2.5% FBS, Heparin (100 µg/mL), GlutaMAX (1% v/v), and Penicillin-Streptomycin (1% v/v).
- **Cell line:** Gibco™ HEK293-F cells adapted to suspension culture with superior transfection efficiencies and high protein expression levels.
- **Equipment:** INFORS HT Multitron triple-stack incubator shaker system with humidity and $CO_2$ control, configured with 25 mm (top and bottom cabinets) and 19 mm (middle cabinets) orbit diameters.
- **Analytical tools:** NC-202 for cell viability and density, YSI biochemical analyzer for glucose and lactate, and OsmoTECH-XT for osmolality measurements.

### Cell culture conditions

Experiments were conducted in 1 L, plain-bottom vented shake flasks with a 300 mL working volume. To ensure uniformity of the culture conditions, multiple flask positions within each Multitron incubator shaker cabinet were tested. The following tables summarize the key culture parameters and conditions used during the two rounds of experiments across the top, middle, and bottom cabinets:

| Culture conditions - Round 1 | | | | | |
|---|---|---|---|---|---|
| **Multitron cabinet** | **$CO_2$ (%)** | **Temperature (°C)** | **Agitation ($min^{-1}$)** | **Orbit diameter (mm)** | **Humidity (% RH)** |
| Top | 8 | 37 | 110 | 25 | 85 |
| Middle | 8 | 37 | 110 | 19 | 85 |
| Bottom | 8 | 37 | 110 | 25 | 85 |

| Culture conditions - Round 2 | | | | | |
|---|---|---|---|---|---|
| **Multitron cabinet** | **$CO_2$ (%)** | **Temperature (°C)** | **Agitation ($min^{-1}$)** | **Orbit diameter (mm)** | **Humidity (% RH)** |
| Top | 8 | 37 | 96 | 25 | 80 |
| Middle | 8 | 37 | 110 | 19 | 80 |
| Bottom | 8 | 37 | 96 | 25 | 80 |

## Results

The experiments demonstrated the Multitron incubator shaker's capacity to support robust HEK293 cell growth and viability across varied conditions. Detailed observations and analyses are as follows:

### Viable cell density (VCD)

HEK293 cells cultured in the Multitron incubator shaker consistently achieved high VCDs across all tested conditions. The average peak density reached approximately 4 million cells/mL by Day 6, as shown in Figure 1. These results demonstrate the capability of the Multitron incubator shaker to provide a supportive environment for robust HEK293 cell growth, regardless of orbit diameter (19 mm or 25 mm).

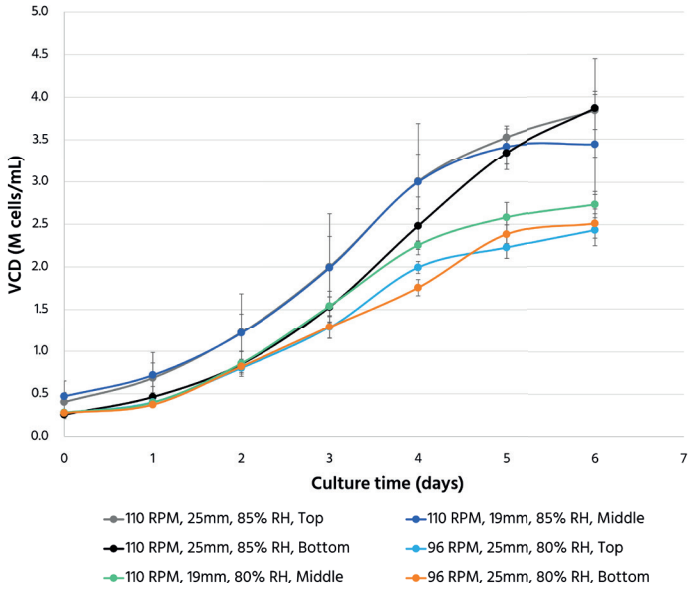



Figure 1. Viable cell density over time.

### Cell viability

Cell viability remained consistently high (>95%) across all cabinets of the Multitron incubator shaker throughout the culture period. The stability of cell viability, even with variations in orbit diameter, underscores the reliability of the incubator's environment. The trends in cell viability are visualized in Figure 2, highlighting the performance of the Multitron incubator shaker in maintaining cell health throughout the culture process.

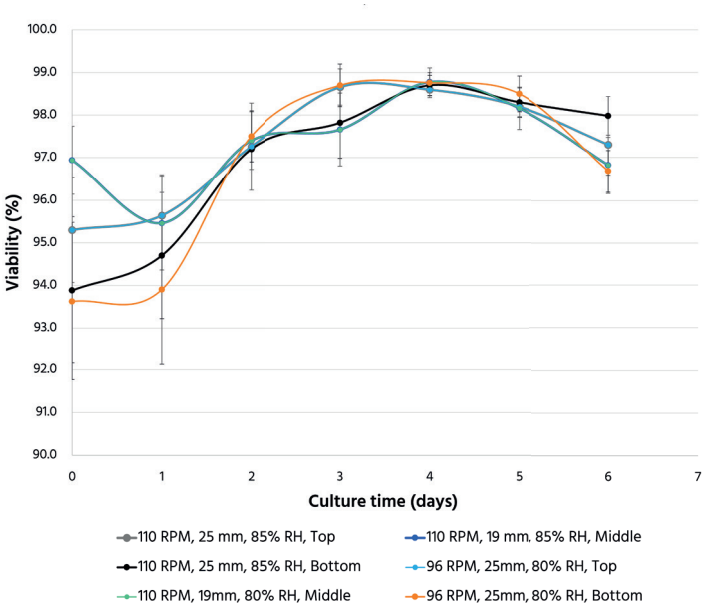



Figure 2. Viability over time.

### Glucose consumption and metabolism

Glucose consumption rates were steady across all experimental conditions. By Day 6, glucose levels were significantly depleted across all cultures, confirming active metabolic processes and ensuring that HEK293 cells maintained healthy metabolic activity, even under different incubation conditions. The data on glucose consumption is illustrated in Figure 3, showing the steady metabolic activity and the absence of significant metabolic disruptions.

### Impact of orbit diameter

The influence of orbit diameter on cell growth and viability was evaluated using the Multitron incubator shaker's adjustable settings. Across cabinets with 19 mm and 25 mm orbit diameters, no statistically significant differences in VCD or viability were observed. Both settings supported robust HEK293 cell growth and viability. While both diameters performed effectively, the 25 mm orbit diameter may offer advantages for specific applications requiring enhanced productivity, such as viral vector production.

### Overall performance

The overall performance of the Multitron incubator shaker was excellent across all measured parameters. The table below presents a summary of the maximum VCD, maximum specific growth rates ($\mu_{max}$), and doubling times, demonstrating the incubator's ability to achieve high cell density and efficient growth while maintaining reproducibility across experimental conditions. These data confirm the Multitron incubator shaker's suitability for scalable bioprocesses involving HEK293 cells in gene therapy applications.

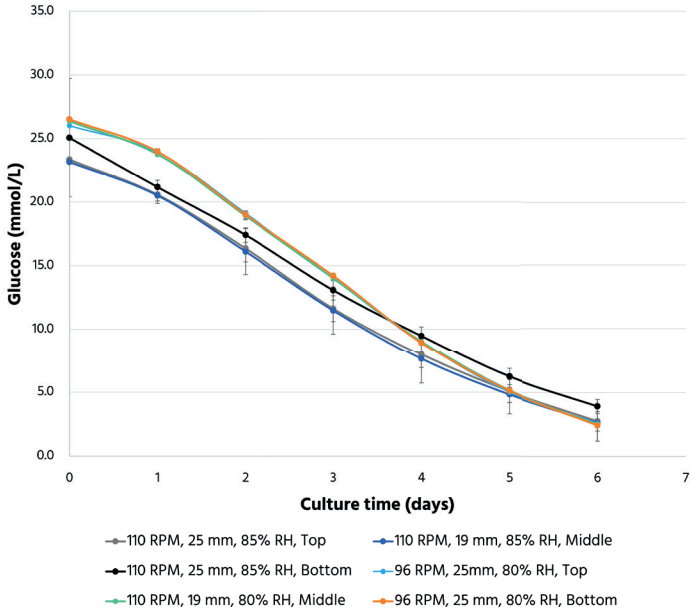



Figure 3. Glucose levels over time.

| Condition | | | | Maximum VCD (M cells/mL) | $\mu_{max}$ (day$^{-1}$) | td (day) |
|---|---|---|---|---|---|---|
| Agitation (min$^{-1}$) | Orbit diameter (mm) | Humidity (% RH) | Cabinet | | | |
| 110 | 25 | 85 | Top | 3.84 | 0.49 | 1.41 |
| 110 | 19 | 85 | Middle | 3.44 | 0.48 | 1.46 |
| 110 | 25 | 85 | Bottom | 3.87 | 0.54 | 1.29 |
| 96 | 25 | 80 | Top | 2.44 | 0.34 | 2.04 |
| 110 | 19 | 80 | Middle | 2.74 | 0.37 | 1.89 |
| 96 | 25 | 80 | Bottom | 2.51 | 0.36 | 1.95 |

Summary of maximum VCD, maximum specific growth rate, and doubling time.

## Conclusion

The Multitron incubator shaker demonstrated excellent performance in improving HEK293 cell cultures for gene therapy applications. The following key factors contributed to the incubator's success:

### Optimal culture conditions

High levels of agitation (110 $min^{-1}$), a larger throw (25 mm), and relative humidity (85% RH) were found to be beneficial for supporting HEK293 cells in suspension culture, especially when aiming to improve metabolic activity and productivity in applications like viral vector production. While no significant differences in viable cell density or viability were observed between 19 mm and 25 mm orbit diameters, the larger orbit diameter aligns more closely with optimal conditions for specific bioprocess applications. This flexibility underscores the Multitron's ability to cater to a wide range of cell culture needs.

### Consistency across positions

The Multitron incubator ensured reproducibility and uniformity of results, as no significant differences were observed in cell growth or viability, regardless of flask positioning (corners or center). This consistency within the same cabinet highlights the incubator's ability to maintain even culture conditions across different flask positions.

### Flexibility for complex applications

The ability to precisely adjust key parameters, such as temperature, $CO_2$, agitation, humidity, and orbit diameter (19 mm and 25 mm), is a standout feature of the Multitron incubator. This flexibility is essential for fine-tuning shear stress conditions and improving HEK293 cell cultures for complex applications like viral vector production, ensuring both scalability and high reproducibility.

### Multi-cabinet advantage

The Multitron's multi-cabinet design offers further advantages, allowing researchers to independently control temperature, $CO_2$, agitation, and humidity for each cabinet. This flexibility supports scalable and tailored experimental setups, making the Multitron incubator an ideal choice for researchers needing precision and adaptability in their HEK293 culture processes.

### Value for gene therapy

The INFORS HT Multitron incubator shaker proves to be a highly reliable and adaptable solution for HEK293 cell culture, particularly in applications requiring high flexibility, precise control, and scalability, such as viral vector production for gene therapy. These capabilities make the Multitron an excellent choice for advancing cell culture processes and improving the production of therapeutic vectors.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