

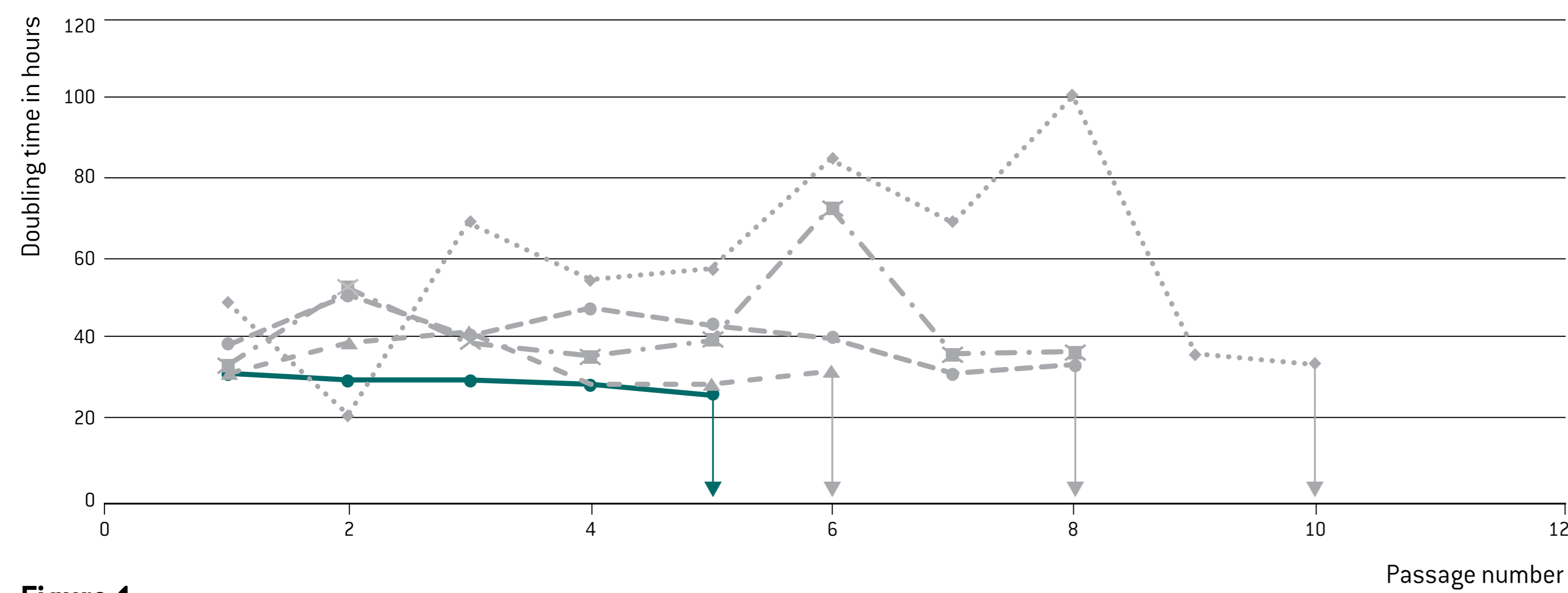
# Evaluation of AAV production in insect cells using chemically defined media

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## Abstract

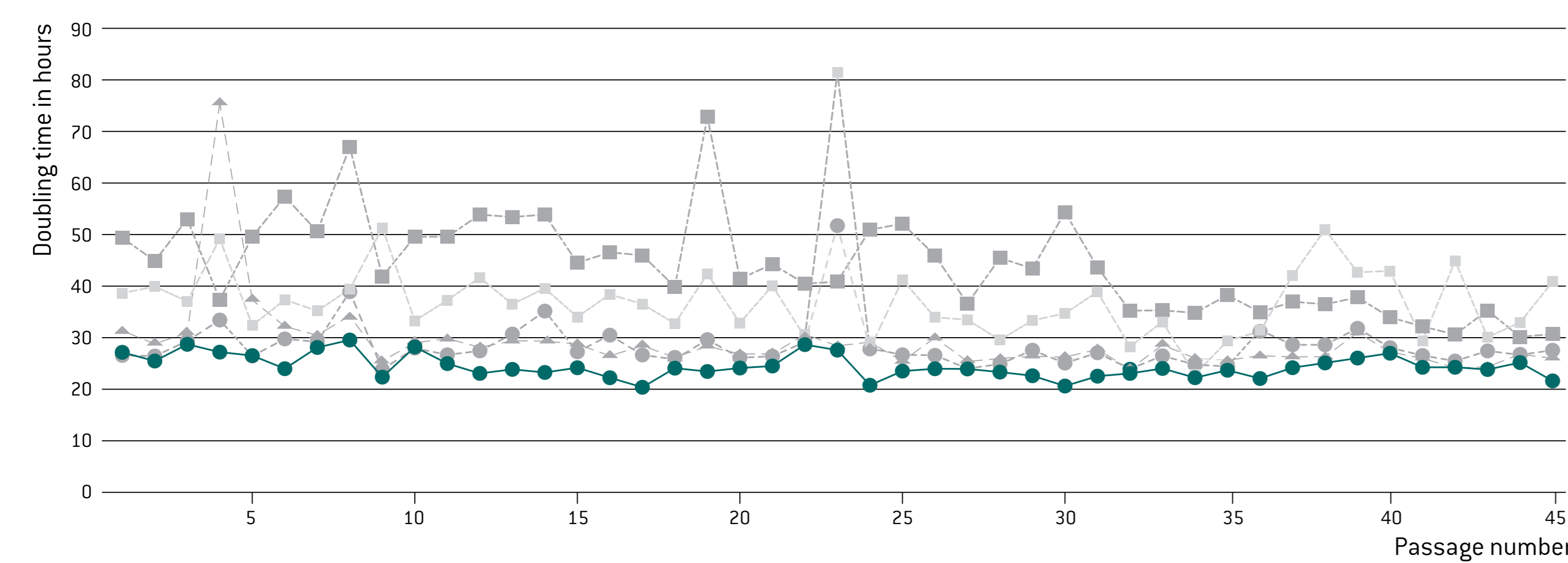
Gene therapy is the fastest growing market amongst all the regenerative medicines. Various viral vectors are used to facilitate the delivery of the gene of interest. Sf9 (*Spodoptera frugiperda*) cells are one of the cell lines predominantly used for Baculovirus and Adeno Associated Virus (AAV) production. This study focuses on evaluation of Lonza's new chemically defined, animal component-free cell culture medium, that supports production of AAV (e.g. rAAV2 and Anc80 - Lonza's proprietary ancestral AAV). TheraPEAK™ SfAAV™ Medium enhances AAV secretion into the extracellular space, easing downstream purification. The absence of ill-defined hydrolysates ensures lot-to-lot consistency, stable doubling time, and increased AAV production compared to other commercially available media formulations.

### Adaptation of Sf9 cells to TheraPEAK™ SfAAV™ Medium



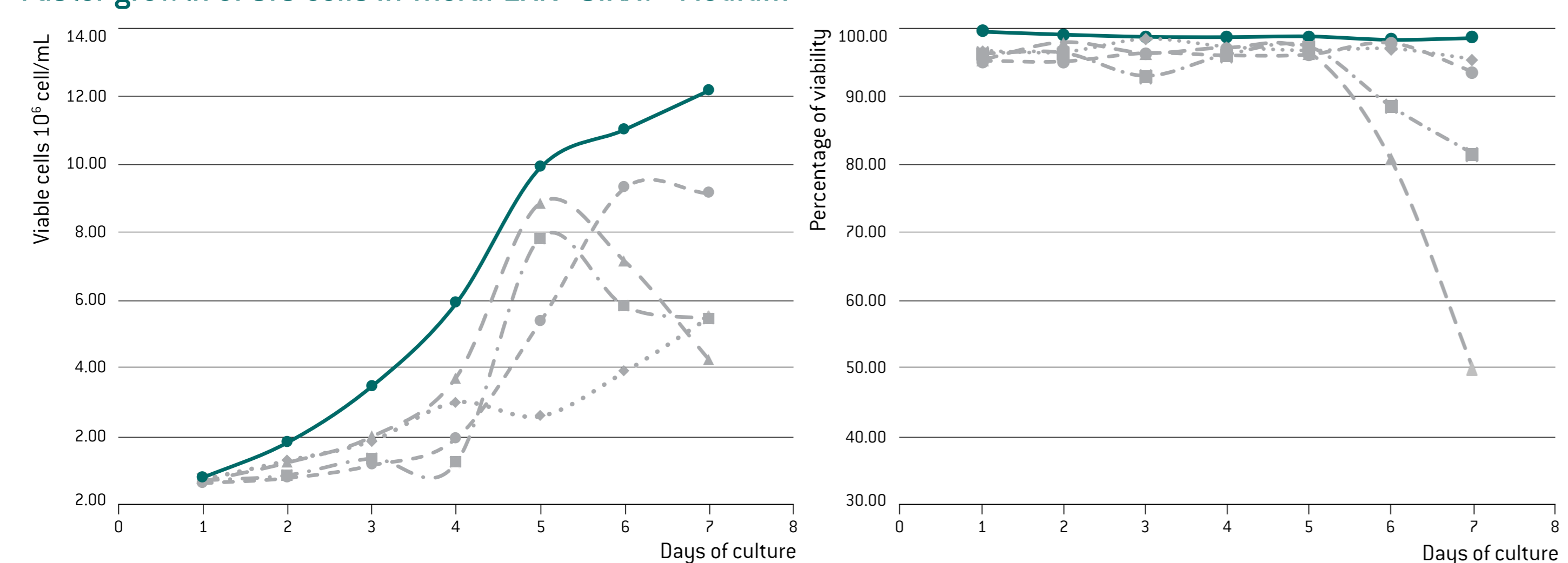
**Figure 1**  
Sf9 cells adapt to TheraPEAK™ SfAAV™ Medium (in green) faster than competitor media (in gray). Data shown represents single replicate shake flasks from a representative experiment. Downward arrows denote when Sf9 cells exhibit doubling time stability and are of sufficient numbers for cell banking.

### Better doubling time of adapted Sf9 cells in TheraPEAK™ SfAAV™ Medium



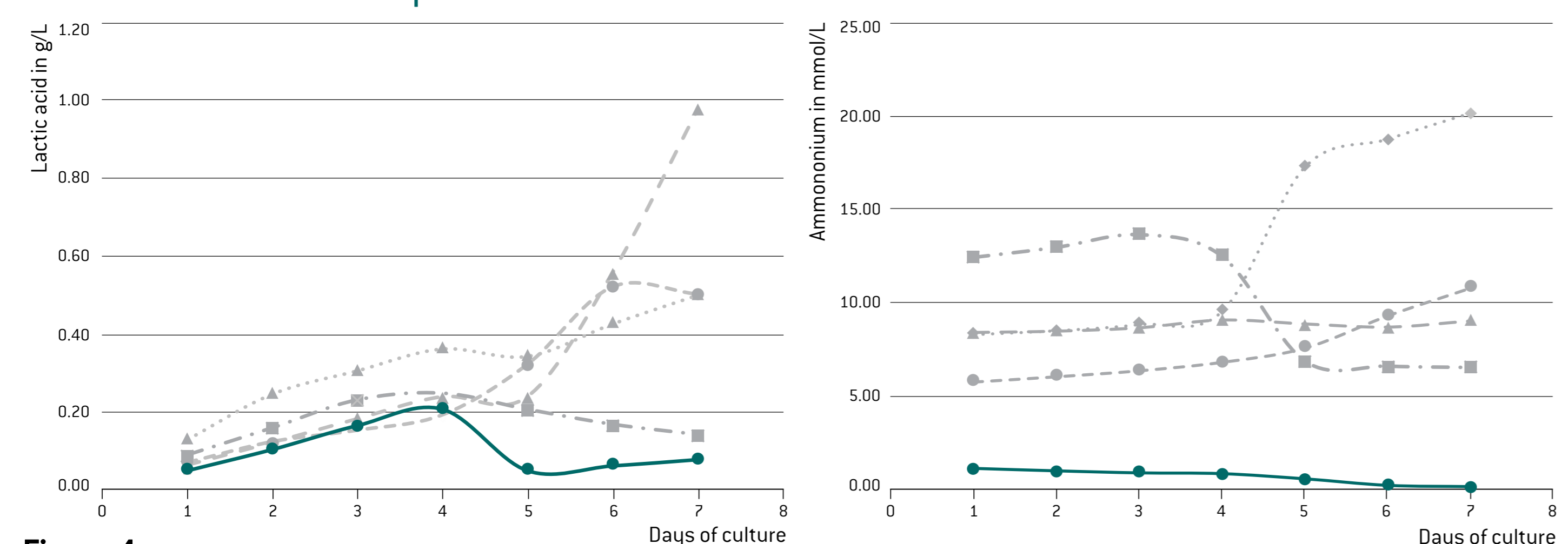
**Figure 2**  
Sf9 cells have better and more stable doubling time over 45 passages in TheraPEAK™ SfAAV™ Medium (in green) than competitor media (in gray) ensuring more consistent Sf9 cell performance over time thereby allowing greater certainty in planning production runs.

### Faster growth of Sf9 cells in TheraPEAK™ SfAAV™ Medium



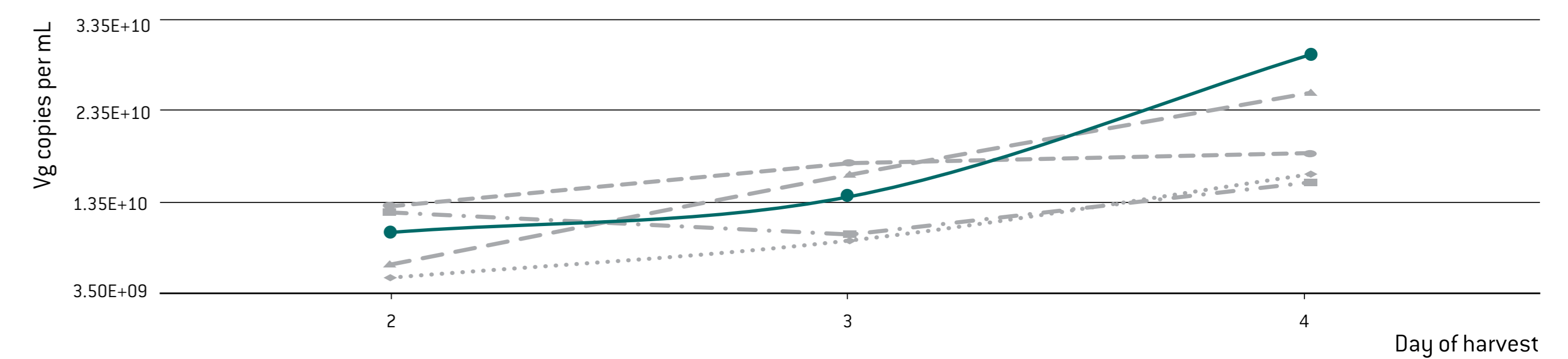
**Figure 3**  
Sf9 cells grow faster and stay viable longer in TheraPEAK™ SfAAV™ Medium (in green) than competitor media (in gray) so researchers are able to initiate baculovirus infections at optimal cell densities (~3x10<sup>6</sup> cells/ml) at least a day earlier than in competitor media.

### Lower lactate and ammonia production with TheraPEAK™ SfAAV™ Medium



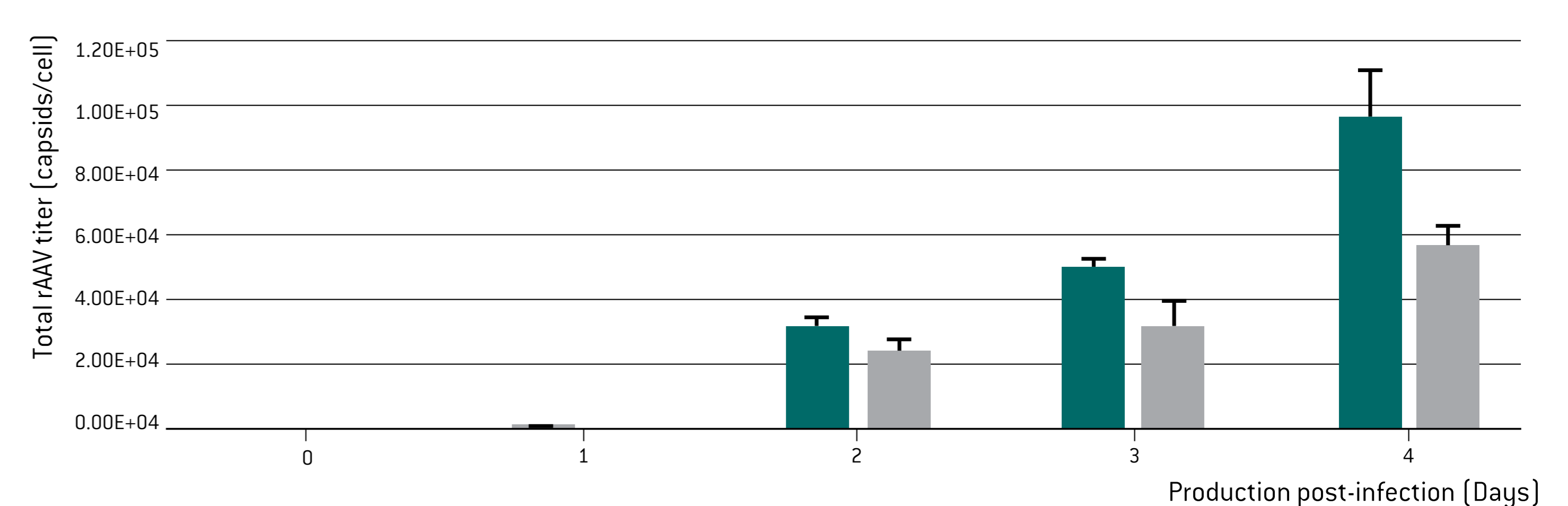
**Figure 4**  
Sf9 cells produce less lactate and ammonia in TheraPEAK™ SfAAV™ Medium (in green) than competitor media (in gray) allowing for healthier virus production.

### Enhanced production of Anc80 in Sf9 cells with TheraPEAK™ SfAAV™ Medium



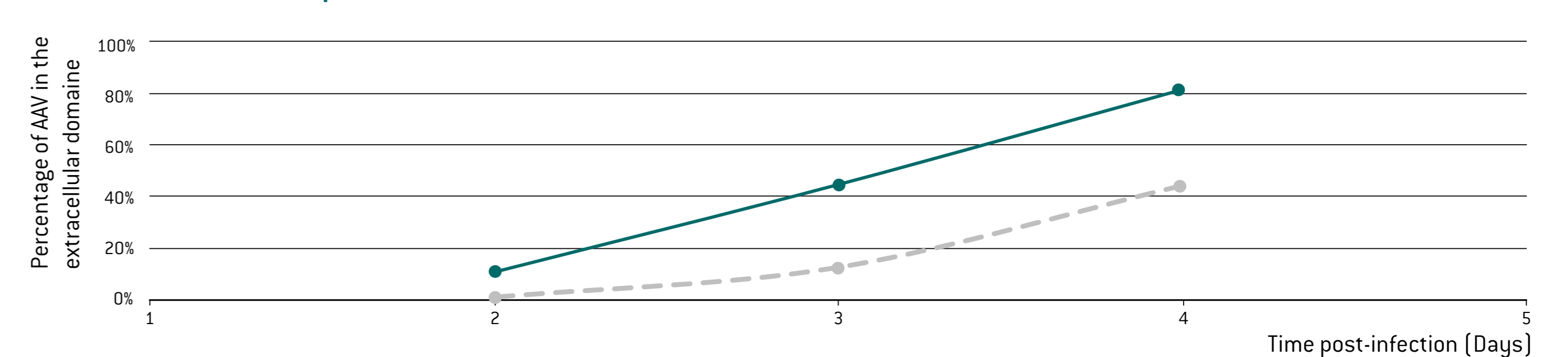
**Figure 5**  
Sf9 cells produced more AAV particles in TheraPEAK™ SfAAV™ Medium (in green) than competitor media (in gray). Greater production of Anc80 AAV in TheraPEAK™ SfAAV™ Medium than other competitor media is observed by 4 days post-infection allowing for greater AAV yield.

### Enhanced production of rAAV2 in Sf9 cells with TheraPEAK™ SfAAV™ Medium



**Figure 6**  
Sf9 cells (adapted to specific medium) grown in bioreactors produce more rAAV2 in TheraPEAK™ SfAAV™ Medium (in green) than leading competitor medium (in grey). Data shown represents triplicate bioreactor runs from a representative experiment and was generated through external collaboration.

### Increase extracellular production of rAAV2 with TheraPEAK™ SfAAV™ Medium



**Figure 7**  
Sf9 cells produce more extracellular rAAV2 in TheraPEAK™ SfAAV™ Medium (in green) than in leading competitor medium (in gray). This allows easier downstream processing and harvesting of AAV particles. Data was generated through external collaboration.

## Conclusion

TheraPEAK™ SfAAV™ is a chemically defined, serum free, animal component-free medium for AAV production. Developed for AAV production in Sf9 cells, TheraPEAK™ SfAAV™ Medium performs better compared to other commercially available medium. The chemically defined formulation, ability to infect earlier, and amount of extracellular AAV produced in this medium allows for more robust, streamlined processing. TheraPEAK™ SfAAV™ Medium has significant benefits for gene therapy applications using AAV in insect cells, including:

- A Allows for rapid adaptation of Sf9 cells from any other medium
- B Provides consistent and high cell growth performance
- C Yields significantly higher AAV production (higher titer)
- D Reduces downstream processing time (more extracellular AAV)
- E Is more regulatory friendly (DMF available)

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