# High-density Pluripotent Stem Cell (iPSC) Expansion in a Single-use Bioreactor Using HiDef® S8

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## Introduction

Historically, human pluripotent stem cell (hPSC) expansion has relied on adherence-dependent culture systems, which involve scaling out to increase yield. Recently, three-dimensional (3D) suspension culture in stirred bioreactors has emerged as a preferred approach due to its capacity to support higher cell densities, improved nutrient and gas exchange, and easier scalability by increasing culture volume[1]. Still, challenges such as aggregate size control, shear effects, and batch-to-batch variability remain significant barriers to efficient suspension culture[2].

In this work, we describe the development of a scalable suspension culture workflow established through a collaboration between Defined Bioscience and Distek that supports large scale, 3D expansion of induced hPSC (iPSC) aggregates in a single-use bioreactor. Central to this workflow is HiDef® S8, a new chemically defined, animal-free suspension medium developed specifically for the efficient expansion and maintenance of hPSCs. Using the 3D workflow described herein, we achieved a 60-fold expansion of hPSC within a single-innoculation five-day run. This demonstrates the potential of HiDef-S8 to facilitate scale-up, reduce cost, and streamline stem cell bioprocessing for both research and therapeutic applications.

## Workflow

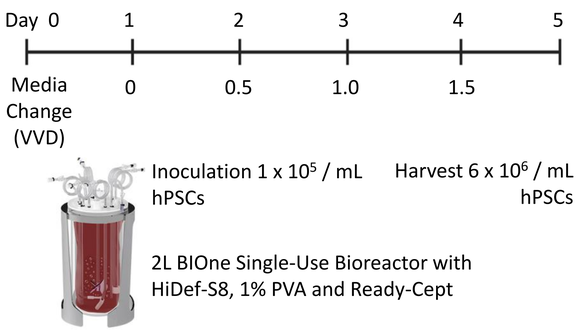


**Figure 1. 3D Suspension Culture Overview.** Five days of suspension culture with media starting at Day 2.

| Parameter | Setpoint |
|---|---|
| Volume | 1200 mL |
| pH | 7.2 +/- 0.1 Cascaded with $CO_2$ |
| Dissolved oxygen | 40% Cascaded with Air and $N_2$ Overlay |
| Temperature | 37°C |
| Overlay Gas | 5% $CO_2$ Enriched Air |
| | 130 sccm |
| Agitation (0-2 hrs) | 170 rpm (5.37 W/m$^3$) Downward axial flow |
| Agitation (2 hrs-5 days) | 250 rpm (17.08W/m$^3$) Downward axial flow |

**Table 1. Operational Parameters for 3D Suspension Culture.**

## Results

The hPSCs cultured in 3D suspension formed viable aggregates with diameters under 200 µm (Fig. 2A). Over the five days of suspension culture, the number of cells increased exponentially in the culture system, resulting in a 60-fold expansion (7.23 x $10^9$ cells) by the fifth day of culture (Fig. 2B).

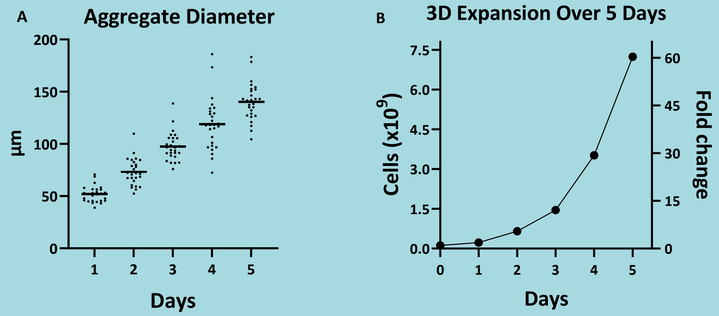


**Figure 2. Three-Dimensional Suspension Culture Is More Efficient**. A. Stirring was optimized for aggregate size to support rapid growth while preserving viability and pluripotency. B. Cell expansion increased exponentially over a five-day run reaching >7 billion cells in 1,200 mL.

This accelerated rate of growth demonstrated a substantial difference in cells produced in 3D suspension over parallel cultures in 2D (Fig. 3A). Moreover, the efficiency of cells produced (number of cells produced per volume of spent medium) was also higher in 3D culture relative to 2D and reached approximately 1.5 million cells generated per mL of medium consumed in 3D suspension culture (Fig. 3A), resulting in a projected supplement cost of US$0.30 per million cells for this line using the Distek-Defined Bioscience platform.

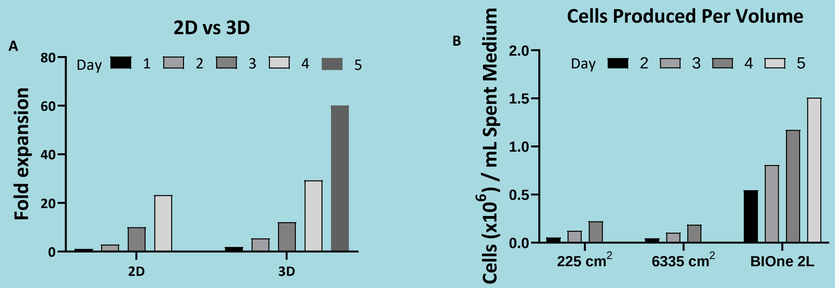


**Figure 3. Higher Fold Expansion In 3D Cultures.** A. Fold expansion comparison between 2D and 3D cultures. B. Number of cells produced per volume of spent media using HiDef-S8 compared to using HiDef-B8 for 2D expansion.

3D aggregates (Fig. 4A) were able to be replated back to 2D using HiDef-B8 medium, acquiring identical morphology to 2D monolayer cells within 24 hours (Fig. 4B). Quantification of pluripotency markers using flow cytometry demonstrated that hPSCs re-plated in 2D and expanded over ten passages co-express surface markers Tra-1-60 and SSEA-4 at unchanged levels (Fig. 4C). Additionally, hPSCs were successfully differentiated to cardiomyocytes in 2D using the CDM3 protocol, with beating cells by the seventh day of culture (Fig .4D).

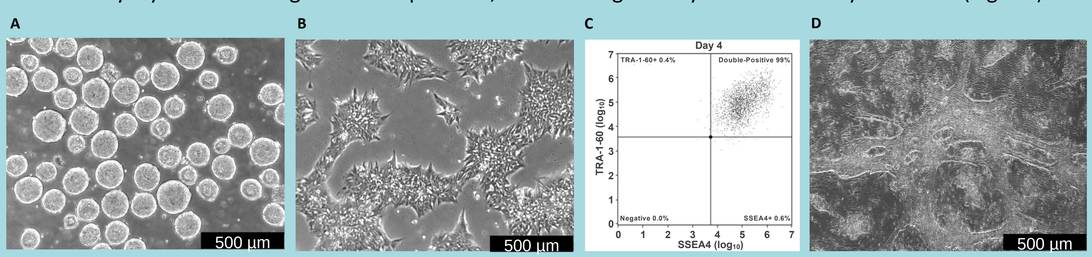


**Figure 4. Suspension Cultures Retain Pluripotency.** A. Aggregate morphology (Day 5). B. Re-plated hPSCs in 2D (Passage 5, Day 2) C. Flow cytometric analysis for Days 4 and 5 suspension cultures hPSC aggregates. C. Cardiomyocyte differentiation (Day 18).

## Conclusions

This study demonstrates that HiDef-S8, with Ready-CEPT® cocktail, supports rapid, high yield, carrier-free expansion of hPSCs in a stirred tank suspension bioreactor. In just five days, seven billion cells were generated in a two-liter BIOne single use bioreactor system, while maintaining high viability and expression of key pluripotency markers. The expanded hPSCs readily re-attached in 2D adherent culture, maintained normal morphology, retained pluripotency after multiple passages and were able to differentiate to cardiomyocytes.

HiDef-S8 was specifically developed to meet the challenges of large-scale, high-density suspension culture and builds on the proven performance of HiDef-B8. Its defined, animal-free composition provides a reliable and scalable platform for seamlessly transitioning hPSC workflows from conventional monolayer cultures to high efficiency bioreactor systems. Distek's robust bioreactor and controller technologies eliminate the need for microcarriers, while Defined Bioscience's HiDef-S8 reduces cost per cell, improves yield per unit of media, and supports the quality requirements of both research and translational applications.

## Future Work

Future work will focus on cGMP manufacturing of HiDef-S8 to support clinical and commercial applications. Additionally, we aim to expand our 3D culture platform to enable the differentiation of hPSCs in suspension to enhance scalability and efficiency for large-scale cell production.

## References

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## Acknowledgments