SARTORIUS

Product Datasheet

Nutri3D hiPSC ACF Medium

Enhanced Formulation for hiPSC Expansion in 2D and 3D Culture Formats

Product Information

Nutri3D hiPSC ACF is an animal component-free (ACF), cGMP-grade medium engineered for reliable pluripotent hiPSC aggregate culture, from small-scale process development to large-scale manufacturing in dynamic suspension bioreactor systems.* The formulation supports rapid, homogeneous aggregate expansion and consistently delivers highly viable cell yields while maintaining pluripotency facilitating controlled differentiation into target germ layers.

Designed to support reproducible hiPSC expansion and downstream manufacturing workflows, the medium accelerates the path from research to scalable cost-efficient bioprocessing.

Features and Benefits

- Optimized for culture and expansion of multiple hiPSC lines
- Two-components: Nutri3D hiPSC Basal Medium and Nutri3D hiPSC ACF Supplement
- Available in bottle and bag formats
- Suitable for research use (RUO) and further manufacturing (coming soon)
- Defined formulation manufactured according to cGMP guidelines
- Animal component-free formulation

Introduction

hiPSCs in 3D Suspension Cultures

Human induced pluripotent stem cells (hiPSCs) hold great potential for patient-specific therapies, reliable disease models, and safer, more predictive drug testing because they can be differentiated into almost any required cell type. To this potential, culture system and media are needed tharealizet expands iPSCs reliably, safely, and at scale. Nutri3D hiPSC ACF is an animal component-free (ACF) medium that supports robust 3D suspension growth of undifferentiated hiPSC as aggregates. Its formulation enables to expand cells without additional supplements (e.g., no extra bFGF) while reducing the need for full medium changes.

Process Scale-Up

Reliable expansion of high-quality iPSCs is essential to generate sufficient cell numbers while maintaining pluripotency for downstream applications. Nutri3D hiPSC ACF is a defined medium for robust, scalable iPSC expansion in 2D and 3D formats. Its composition enables controlled, reproducible culture conditions while maintaining high pluripotency and stable karyotype. It is compatible with cells previously cultured in NutriStem® hPSC XF Medium with CellGenix® growth factors simplifies transition. The medium performs reliably across 2D cultures, shake flasks, Ambr® systems, vertical-wheel bioreactors, and large-scale platforms, supporting workflows from research to clinical applications.

Performance

Growing demand for large-scale 3D manufacturing of hiPSC-derived cells puts pharmaceutical manufacturers under pressure to control process costs and timelines, while improving output. Nutri3D hiPSC ACF supports single-medium workflows from the initial 2D seeding to 3D bioreactor expansion, reducing process development and transfer time while helping to increase yields and shorten batch cycles. Tested across multiple independent hiPSC lines, it delivers homogeneous aggregate expansion and consistently delivers high viable cell yields with maintained pluripotency and differentiation potential.

Quality

Maintaining genetic stability and pluripotency in iPSC cultures is critical for reliable downstream differentiation and safe therapeutic applications. Nutri3D hiPSC ACF supports stable hiPSC cultures during routine single-cell passaging and high-density expansion, helping reduce passaging-related cellular stress. Manufactured under cGMP guidelines with tightly controlled raw materials, it offers higher lot-to-lot consistency. Each lot is performance tested in a culture assay to ensure consistent performance for iPSC-based therapies and research, with a DMF in preparation to support regulatory-facing workflows.

Pluripotent Stem Cells Workflow

Cell sourcing	Reprogramming & engineering	**Expansion**	Differentiation target lineage

Nutri3D ACF Medium - Your Scalable Solution

2D Seed train hiPSCs monolayer	›	Small-scale hiPSCs 3D culture as aggregates	›	Mid-scale hiPSCs 3D culture	›	Commercial production

Nutri3D hiPSC ACF Medium - Addressing Industry Needs

Feature	Challenge	Nutr3D hiPSC ACF Benefits
Scalability	Transition from 2D seed train culture to 3D aggregate expansion is complex	Single-medium workflow supports 2D seed train culture and scalable 3D aggregate expansion
Performance	Limited expansion and low viable cell yields can impact performance	Efficient iPSC aggregate generation with high viable cell yield and fold expansion from low seeding densities.
Consistency	Variable aggregate size and growth reduce reproducibility and increase costs	Uniform aggregates and defined formulation support consistent, cost-efficient 3D workflows
Cell quality	Expansion can compromise pluripotency or downstream differentiation capacity	High pluripotency marker expression and stable karyotype supports efficient downstream lineage workflows
Regulatory readiness	Uncontrolled media or documentation gaps delay drug development	ACF formulation, qualified raw materials, ISO 13485 QMS and cGMP manufacturing, DMF in preparation

Application Data - 2D iPSC Culture

2D hiPSC Expansion Benchmark

Nutri3D hiPSC ACF supports robust 2D expansion of hiPSC cells under defined, animal component-free conditions. Its suitability was benchmarked against commercially available ACF and XF competitor media intended for 2D iPSC culture. Cultures were maintained for multiple passages using two independent human iPSC lines on Laminin-521 (LN-521) and one line on Vitronectin (VTN).

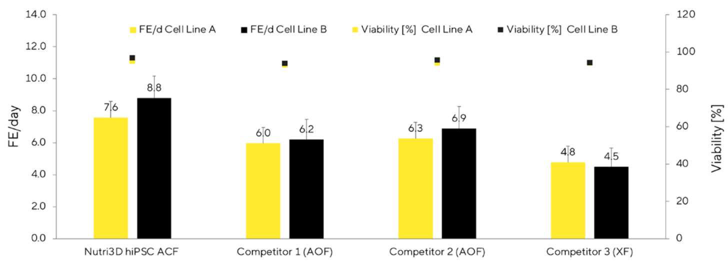


Figure 1: *Average of fold expansion per day (FE/d) for 4 passages for two iPSC cell lines (n=2) on LN-521 (0.5 µg/cm²). Cells were seeded at 7,000 - 12,000 cells/cm² and dissociated with recombinant Trypsin-EDTA every 3-4 days.*

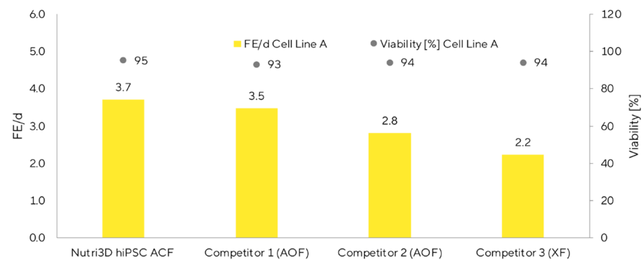


Figure 2: *FE/d (4 passages) of one hiPSC line cultured on VTN-coated wells (0.5 µg/cm²). Culture conditions were equivalent to those used for LN-521 cultures.*

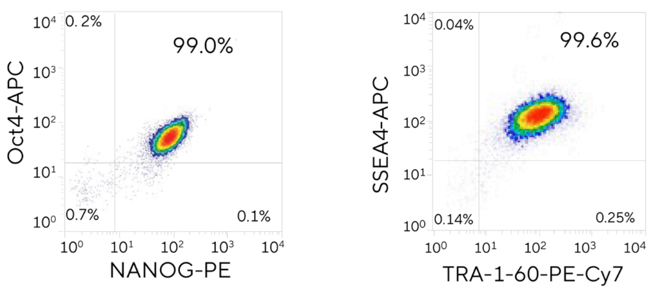


Figure 3: *High pluripotency marker expression maintained after expansion in Nutri3D hiPSC ACF on LN-521.*

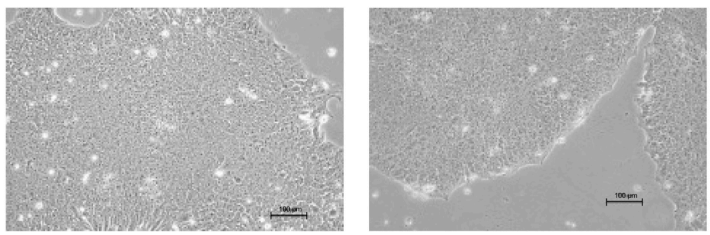


Figure 4: *Representative micrographs showing compact iPSC colonies with uniform morphology in 2D monolayer culture (P3). Left: LN-521; right: VTN. 100×; scale bar: 100 µm*

Nutri3D hiPSC ACF delivers strong 2D performance on recombinant matrices outperforming 2D competitor media, enabling complete ACF single-medium workflows from 2D seed train culture to 3D aggregate expansion.

Application Data - 3D Culture of iPSC

3D hiPSC Aggregate Expansion – Vertical Wheel Bioreactor

Nutri3D hiPSC ACF is designed for efficient 3D aggregate culture of hiPSCs in bioreactors. To evaluate its performance, hiPSCs were cultured as 3D aggregates in a 100 mL PBS-MINI® bioreactor and benchmarked against a commercially available competitor medium intended for 3D expansion under identical, controlled conditions. All cultures were run without shear protectant (PF-68). 2D seed train was performed on LN-521 using the corresponding 2D medium for each system before transfer to 3D suspension culture.

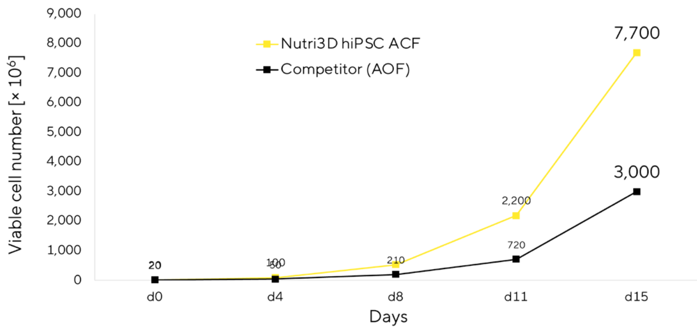


Figure 5: *Significantly higher accumulative viable cell number for 3D aggregate culture for hiPSCs cultured in Nutri3D hiPSC ACF compared to competitor.*

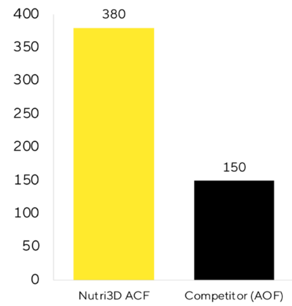


Figure 6: *Cumulative FE at day 15*

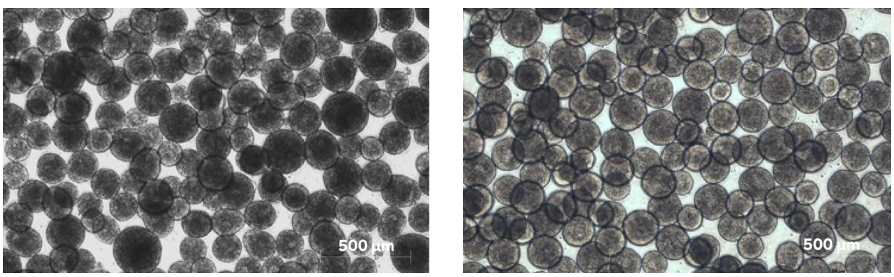


Figure 7: *Representative micrographs (scale bar: 500 µm, 100×) at day of 3D suspension iPSC aggregates in Nutri3D at P1 (left) and P4 (right) showing uniform, round aggregate morphology.*

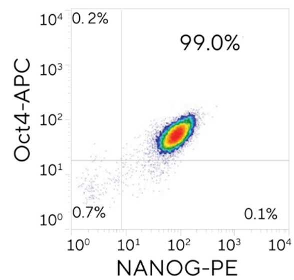


Figure 8: *hiPSCs maintain pluripotency marker expression (>97%) throughout culture.*

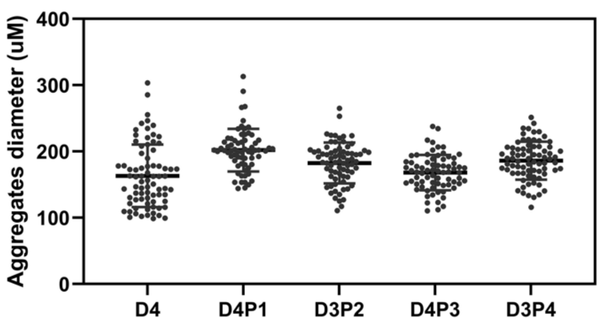


Figure 9: *Uniform size distribution of hiPSC aggregates cultured with Nutri3D hiPSC ACF over multiple passages (day 4 of each passage).*

From 20 million to ~7.7 billion hiPSCs in 15 days – Nutri3D hiPSC ACF yields ~2.5× more total viable cells than competitor medium under identical conditions with maintained pluripotency and healthy aggregates.

3D hiPSC Aggregate Expansion - Shake Flasks

Nutri3D hiPSC ACF was evaluated for 3D aggregate expansion in Erlenmeyer shake flasks against two commercial 3D iPSC media using three independent hiPSC lines. Culture conditions followed each manufacturer's instructions, including the optional addition of shear protectants, such as PF-68. Cells were pre-expanded in 2D using the corresponding 2D medium.

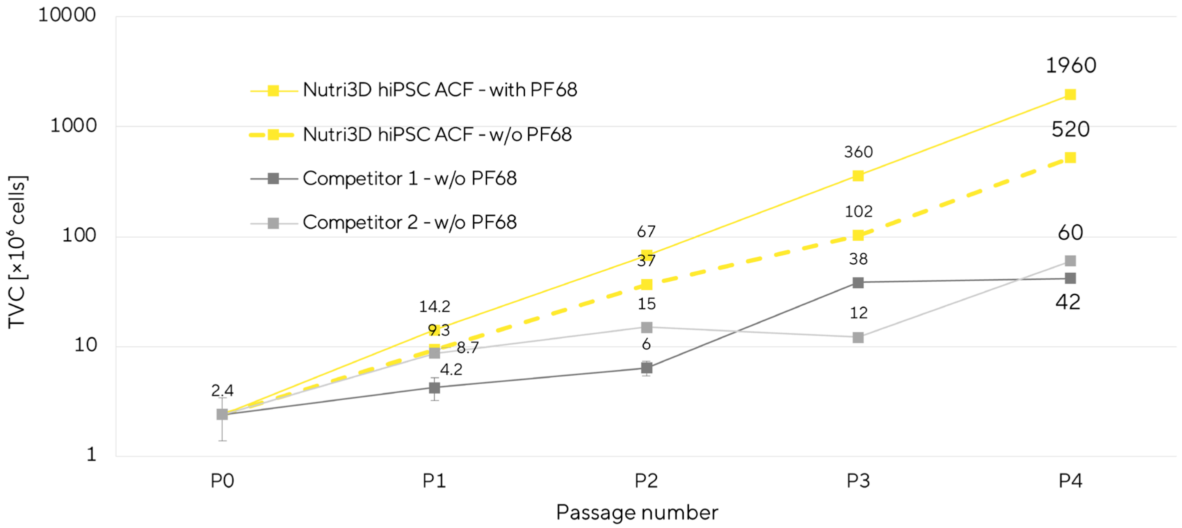


Figure 10: *Total viable cell (TVC) number of one iPSC line (N = 3, mean value) expanded as 3D aggregates in 20 mL shake flasks after 2D seeding on LN-521. Nutri3D hiPSC ACF exceeded competitors without shear protectant and further improved expansion upon optional addition.*

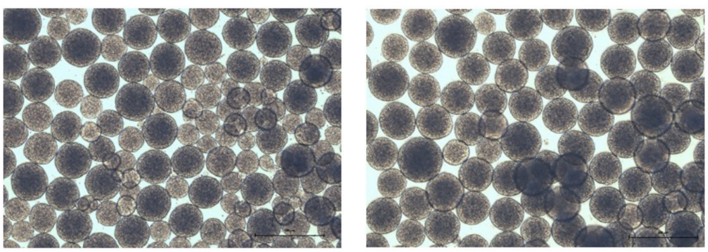


Figure 11: *Representative micrographs of iPSC aggregates after P3 uniform showing healthy morphology (scale bar: 500 µm x40); with PF-68 (left) and w/o PF-68 (right)*

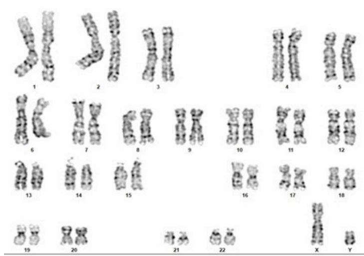


Figure 12: *Karyotype confirms genomic stability of iPSC aggregates in Nutri3D*

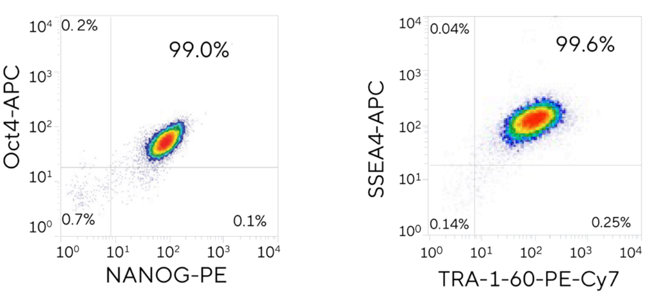


Figure 13: *Representative FACS profiles. Pluripotent markers for all three iPSC cell lines ≥ 95%.*

Nutri3D hiPSC ACF supported robust 3D aggregate expansion in shake flasks with or without shear protectant, outperforming comparator media under IFU-based conditions and reducing the need for additional supplements or surfactants.

3D hiPSC Aggregate Expansion - Ambr® 250 Modular

Nutri3D hiPSC ACF supports scalable 3D expansion of hiPSC aggregates in stirred-tank bioreactor (STBR) workflows. Performance was assessed using the Ambr® 250 Modular system with a commercial hiPSC line for 5 days with partial media exchange. The culture was supplemented with a surfactant to support aggregate suspension under stirred bioreactor conditions.

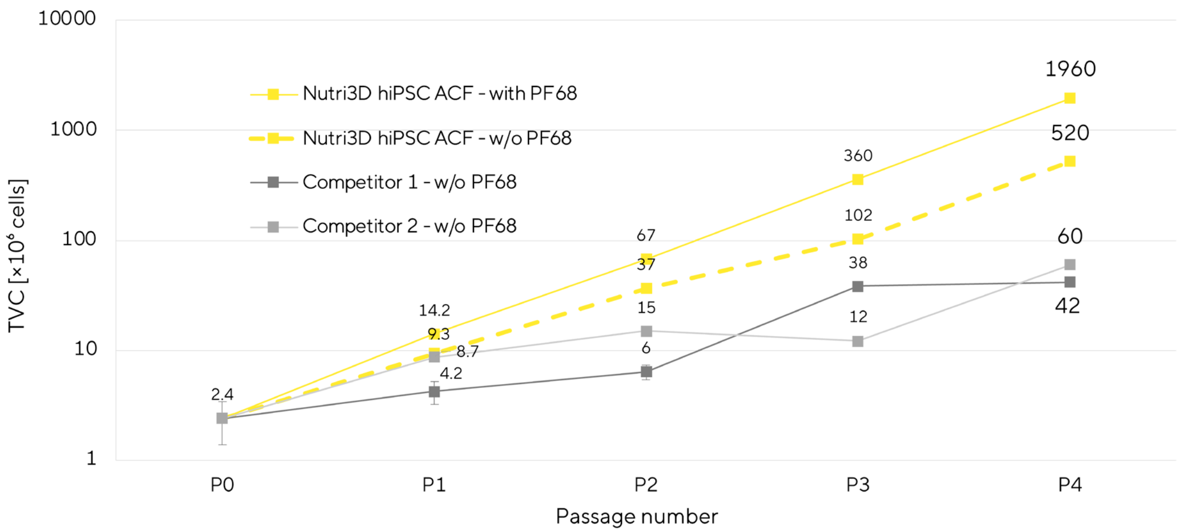


Figure 14: *Total viable cell (TVC) number of one iPSC line (N = 3, mean value) expanded as 3D aggregates in 20 mL shake flasks after 2D seeding on LN-521. Nutri3D hiPSC ACF exceeded competitors without shear protectant and further improved expansion upon optional addition.*

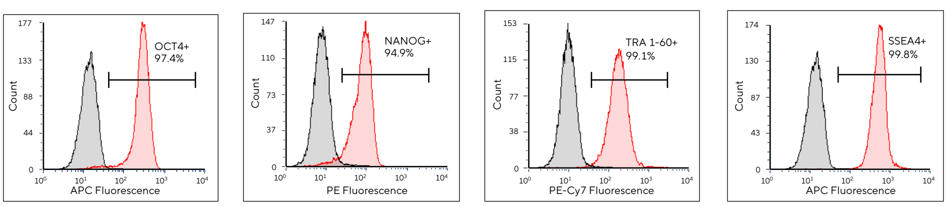


Figure 15: *Figure 99: 3D hiPSC aggregates remained positive (>94%) for essential pluripotency markers at the end of cultivation (n = 2). From left to right: OCT4, NANOG, TRA-1-60, and SSEA-4.*

Nutri3D hiPSC ACF provides a scalable medium solution for 3D hiPSC aggregate expansion in stirred-tank bioreactor workflows, supporting high viable cell yields while maintaining essential hiPSC quality attributes.

Application Data - Differentiation

Trilineage differentiation competence of cultured hiPSCs

Functionality of hiPSCs cultured in Nutri3D hiPSC ACF from the different 3D culture systems was confirmed using a commercial trilineage differentiation kit (StemMACS™), with differentiation into ectodermal, mesodermal, and endodermal lineages assessed by flow cytometry for lineage-specific markers. Pluripotency status before and after differentiation was assessed to confirm loss upon lineage commitment.

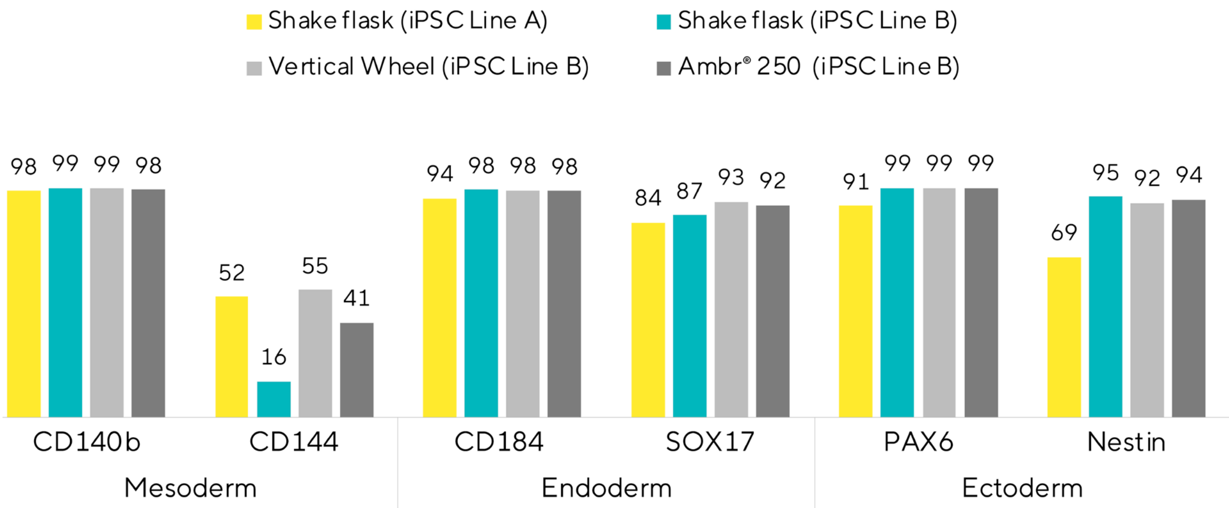
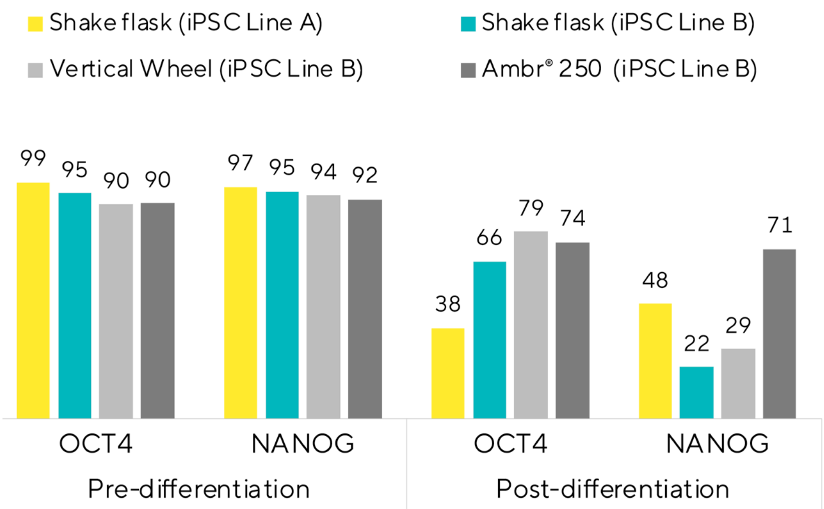


Figure 16: *Flow cytometry analysis of lineage-associated markers after trilineage differentiation, shown as percentage of marker-positive cells.*



hiPSCs expanded in Nutri3D ACF Medium retain robust trilineage differentiation capacity, with high differentiation efficiency across all three germ layers.

Figure 17: *Flow cytometry shows efficient downregulation of OCT4 and NANOG post-differentiation.*

Directed differentiation of hiPSCs into cardiomyocytes

Successful directed cardiac differentiation of 3D hiPSC aggregates expanded in shake-flask culture was demonstrated across two independent hiPSC lines. Differentiation was performed using chemically defined CDM3 medium and a modified Burridge et al. protocol (Nat Methods, 2014) adapted for 3D aggregate culture.

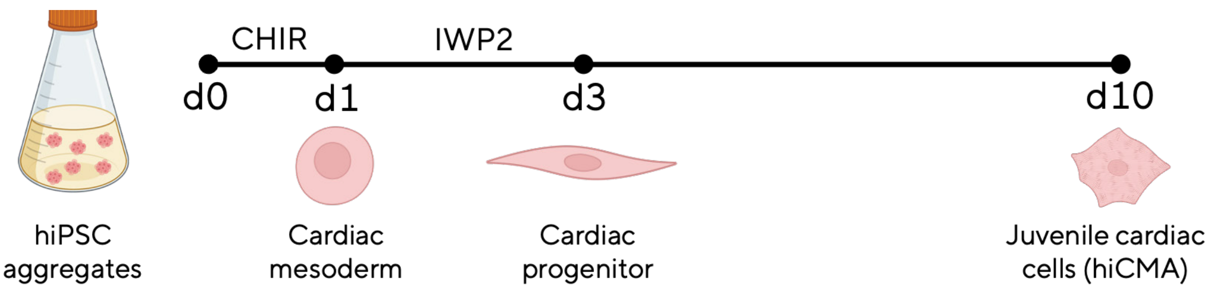


Figure 13: *Workflow for directed cardiac differentiation of Nutri3D hiPSC ACF-expanded hiPSC aggregates each for two iPSC lines.*

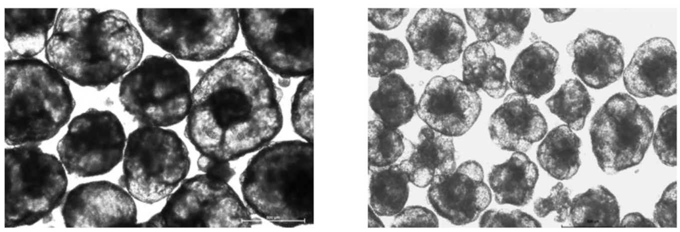


Figure 19: *Representative micrographs at day 10 (scale bar: 500 µm; 40×) of cardiac-differentiated aggregates derived from two hiPSC lines, showing compact aggregate morphology (viability >99%).*

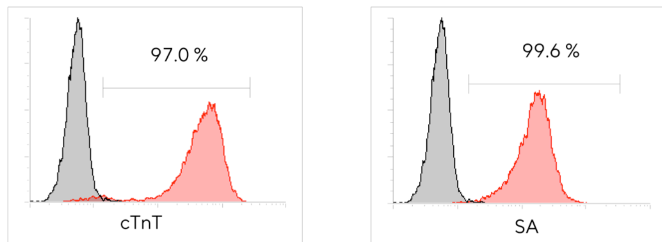


Figure 20: *Highly efficient definitive endoderm differentiation. FACS analysis (day 4) shows >92% of cells positive for specific markers for the two iPSC lines.*

Nutri3D hiPSC ACF-expanded hiPSCs were successfully differentiated into cardiomyocytes, facilitating a seamless transition from 3D expansion to downstream workflows.

Ordering Information

Item	Volume	Packaging	Cat. No
Nutri3D hiPSC ACF basal	500 mL	Bottle	O5-F3F1009-1A
Nutri3D hiPSC ACF basal	5 L	Bag	O5-F3F1009-1-5L
Nutri3D hiPSC ACF basal	10 L	Bag	O5-F3F1009-1-10L
Nutri3D hiPSC ACF supplement mix (x100)	5 mL	Bottle	O5-F3F1012-1H
Nutri3D hiPSC ACF supplement mix (x100)	50 mL	Bottle	O5-F3F1012-1E
Nutri3D hiPSC ACF supplement mix (x100)	100 mL	Bottle	O5-F3F1012-1B

Related Products

Item	Cat. No
NutriFreez® D5 Cryopreservation Medium	05-715-1
NutriFreez® D10 Cryopreservation Medium	05-713-1
NutriFreez® D10 Cryopreservation Medium Phenol-Red Free	05-714-1
NutriStor® Cold Storage Solution	05-F3F3001-1
Recombinant Trypsin Solution	03-078-1
Recombinant Trypsin-EDTA Solution	03-079-1
EDTA Solution 0.05%, 100 mL	03-015-1B
EDTA 0.5 M, 100mL	01-862-1B
Ambr® 250 Modular Mammalian Low-Shear Vessel	001-5A33

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

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Status: 07 | 2026