hPSC CULTURE

From Sourcing to Maintaining Human Pluripotent Stem Cells

STEMCELL™ TECHNOLOGIES

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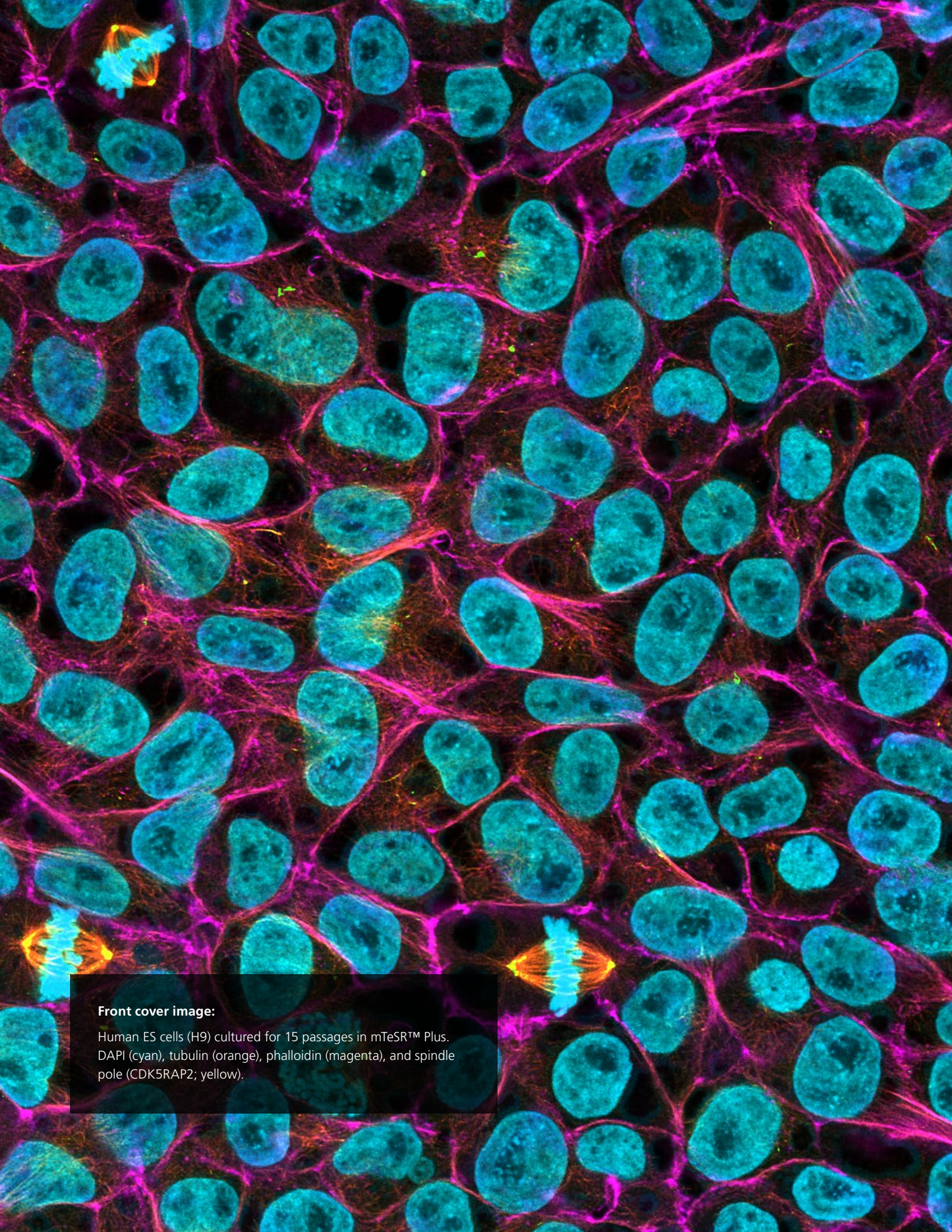
PDF



Front cover image:

Human ES cells (H9) cultured for 15 passages in mTeSR™ Plus. DAPI (cyan), tubulin (orange), phalloidin (magenta), and spindle pole (CDK5RAP2; yellow).

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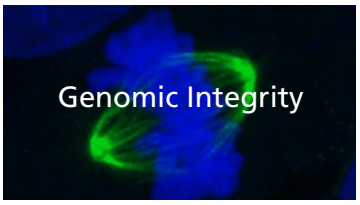
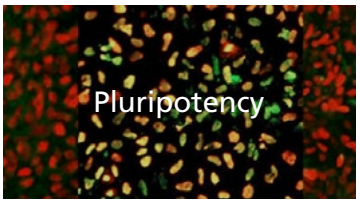
Cell Quality

Managing human pluripotent stem cell (hPSC) line variability and health during maintenance can be challenging. Proper reporting and standardized quality control measures can help limit variability and ensure that relevant, reproducible findings are shared. Learn about important cell quality attributes of hPSCs below, and find out how you can assess and maintain high-quality hPSC cultures.



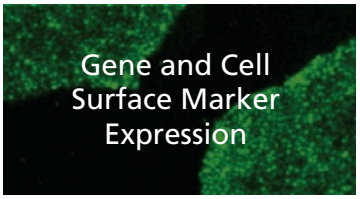
hPSCs frequently acquire abnormalities in a non-random and sporadic manner. The most commonly affected chromosomes in hPSCs include 1, 8, 10, 12, 17, 18, 20, and X. Understanding and detecting these changes is key to ensuring the safety and efficacy of future therapies derived from hPSCs.

For the hPSC Genetic Analysis Kit, please see page 13.



While common hPSC markers should be expressed by undifferentiated hPSCs, they are not necessarily markers of "pluripotency." Therefore, it is essential to validate pluripotency in a functional manner by differentiating to the three germ layers: ectoderm, mesoderm, and endoderm.

For the STEMdiff™ Trilineage Differentiation Kit and the hPSC Trilineage Differentiation qPCR Array, please see page 14.

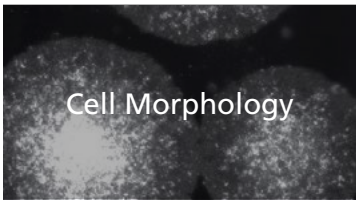


hPSCs are characterized by specific cell-surface markers, such as the glycolipid antigens SSEA3 and SSEA4, as well as the glycoprotein antigens TRA-1-60 and TRA-1-81. Transcription factors OCT3/4, SOX2, and NANOG are also highly expressed and are key elements in the "pluripotency network."

For antibodies to test for gene and cell surface marker expression, please see page 15.

There is substantial latency in the iPSC reprogramming process, with epigenetic aberrations in early passages reflecting transient epigenetic memory from the cell types that the iPSCs are derived from. Using only iPSCs that are fully reprogrammed and characterized is important to avoid aberrations.

For RSeT™ and NaïveCult™, please see pages 26 and 27.



Under low magnification, healthy and high-quality hPSCs should exhibit the following characteristics: round, defined borders; dense, phase-bright centers; tight cellular packing with a high nuclear-to-cytoplasmic ratio; and prominent nucleoli.

For training in hPSC maintenance, including how to assess the morphology of hPSCs, please see page 34.

The ISSCR's Standards for Human Stem Cell Use in Research

As a company of Scientists Helping Scientists, STEMCELL Technologies is a passionate advocate for standardizing hPSC data reporting and quality control measures, limiting experimental variability, and ensuring that relevant and reproducible findings are shared. The ISSCR's Standards for Human Stem Cell Use in Research document represents a critical step toward these goals.[1] The products in this brochure can help you to achieve high-quality results that align with the Standards. Visit **www.stemcell.com/ISSCR-Standards** to learn how STEMCELL can support you in following these important guidelines.

Wallchart

Reporting Practices for Publishing Results with hPSCs

Cell Sourcing: hPSC Lines

Healthy Control Human iPSC Lines: SCTi003-A and SCTi004-A

Start with High-Quality Cells

Derived from peripheral blood mononuclear cells (PBMCs), the Healthy Control Human iPSC Lines, SCTi003-A (Catalog #200-0511) and SCTi004-A (Catalog #200-0769) have undergone extensive quality control procedures and been validated with STEMCELL products for applications including culture scale-up or differentiation to multiple cell types in both 2D and organoid models.

Note: For research use or in vitro laboratory-based tissue culture work only. Not approved for application into humans under any circumstances.

Trilineage Differentiation Capabilities

STEMCELL's iPSC lines are validated for use with a wide range of products, including many STEMdiff™ differentiation kits.

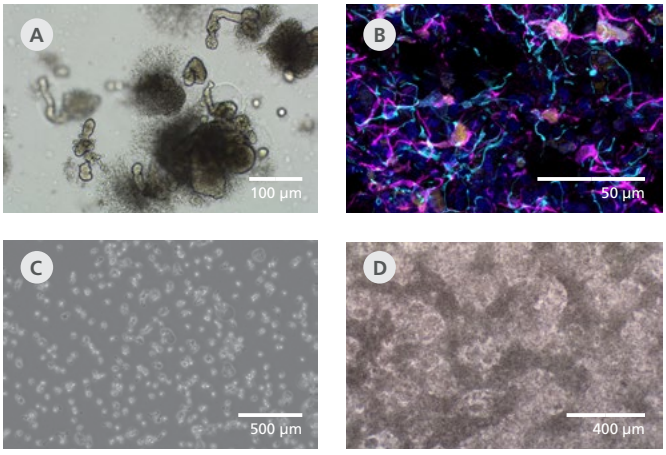


Figure 1. STEMCELL's hPSCs Differentiate into All Three Germ Layers

SCTi003-A iPSCs are validated with many STEMdiff™ differentiation and maturation kits. (A) They can be differentiated to intestinal spheroids and embedded in Matrigel® domes for maturation into human intestinal organoids (shown at Day 13) with the STEMdiff™ Intestinal Organoid Kit, and passaged and expanded with STEMdiff™ Intestinal Organoid Growth Medium. (B) Neural Organoids stained for DAPI (blue), MAP2 (magenta), NEUN (yellow), and GFAP (cyan) can be differentiated using the STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit and maintained with the STEMdiff™ Neural Organoid Maintenance Kit. (C) Microglia with visible processes and small cytoplasmic-to-nuclear ratios can be generated via a hematopoietic progenitor cell intermediate using the STEMdiff™ Hematopoietic Kit, and further differentiated with the STEMdiff™ Microglia Differentiation and Maturation Kits. (D) Ventricular cardiomyocytes can be generated with the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit to form a monolayer that exhibits beating behavior. For more information about STEMdiff™ products, visit **www.stemcell.com/stemdiff**.

Demographic Information

STEMCELL collects donor demographic information ethically, using consent forms and protocols approved by either an institutional review board (IRB), the Food and Drug Administration (FDA), the U.S. Department of Health and Human Services, and/or an equivalent regulatory authority. Donations are performed in the United States in compliance with applicable federal, state, and local laws, regulations, and guidance.

Why Use iPSC Lines from STEMCELL?

- Meet regulatory requirements for academic and/or commercial purposes with ethically sourced human iPSCs
- Trust in extensive quality control that meets or exceeds industry standards[2]
- Enhance research transparency, and ethical and biological conformity, by using a hPSCreg®-certified cell line
- Confidently integrate hPSCs into your workflow with TeSR™- and STEMdiff™-compatible cell lines

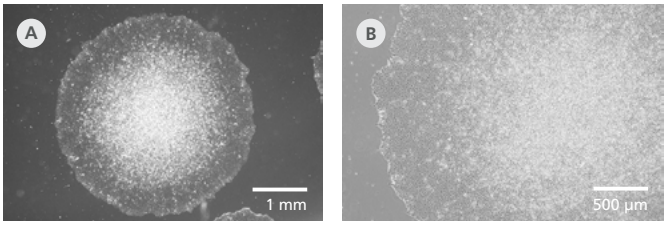


Figure 2. STEMCELL's hPSCs Exhibit High-Quality Morphology

Cryopreserved cells from STEMCELL's line SCTi003-A were thawed and maintained in mTeSR™ Plus on Corning® Matrigel® Matrix. (A) The resulting iPSC colonies have densely packed cells and show multi-layering when ready to be passaged. (B) Cells retain prominent nucleoli and high nuclear-to-cytoplasmic ratios.

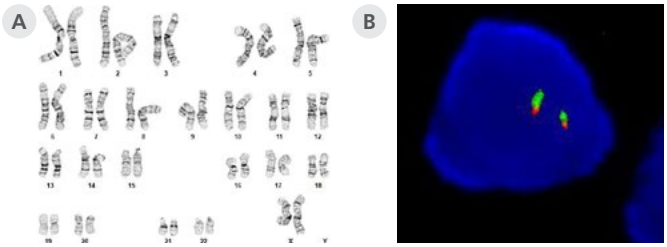


Figure 3. STEMCELL's hPSCs Maintain a Normal Karyotype

(A) G-T-L banding for thawed cells at p26 (n = 20) shows a normal karyotype with no evidence of clonal abnormalities at a resolution of 450 - 550 G-bands per haploid genome. (B) Fluorescent in situ hybridization in a p26 iPSC using probes for 20p11 (green) and 20q11.21 (red). 94% of cells examined displayed two sets of two probe signals, indicating no aneusomy of chromosome 20 (n = 200).

Product Information
Additional data on the SCTi003-A line
www.stemcell.com/scti003-a

Resource
Frequently Asked Questions on iPSCs
www.stemcell.com/ipsc-faq

iPSCdirect™: A Source of Ready-to-Use hPSCs in a Single-Cell Format

Speed up your research with thaw-to-use, single-cell format iPSCdirect™ cells (Catalog #100-1028 and #200-0510). These high-density cryopreserved iPSCs are fully characterized and eliminate the need for hPSC maintenance, saving you both time and money. Derived from the SCTi003-A cell line, iPSCdirect™ has undergone the same extensive quality control procedures that meet or exceed industry standards. Each vial contains 10 million viable cells, which are ready for use in downstream applications, such as differentiation using STEMdiff™ media products as early as 24 hours post-plating.

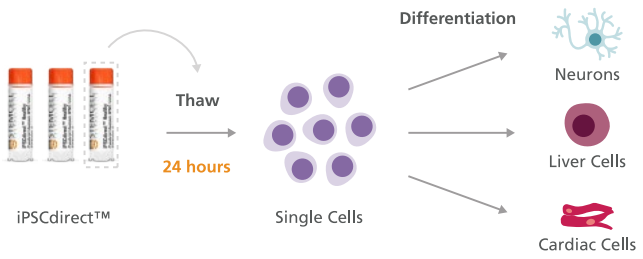


Figure 4. iPSCdirect™ Fast-Tracks hPSC Workflows

iPSCdirect™ cells can be thawed and plated into mTeSR™ Plus (Catalog #100-0276) supplemented with CloneR™2 (Catalog #100-0691) and incubated overnight according to product instructions. Seeding density recommendations can be found in the Product Information Sheet. After 24 hours, cells are ready for STEMdiff™ or customized monolayer workflows.

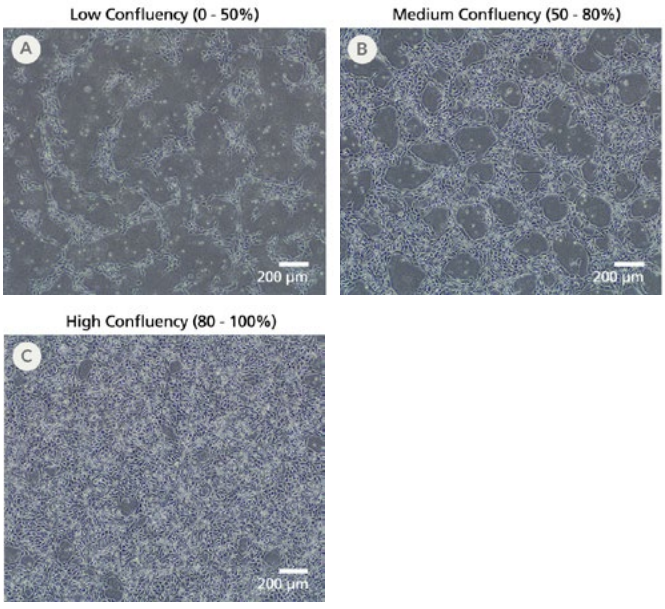


Figure 5. iPSCdirect™ Cells Can Be Seeded to Reach a Range of Confluencies After 24 Hours

To reach the desired confluency for downstream experiments, thaw and plate iPSCdirect™ cells into mTeSR™ Plus with CloneR™2 (Catalog #100-0276 and #100-0691) at the densities recommended in the Product Information Sheet. These representative examples of (A) low confluency, (B) medium confluency, and (C) high confluency were cultured after thaw on Corning® Matrigel® hESC-Qualified Matrix and imaged at a magnification of 4X.

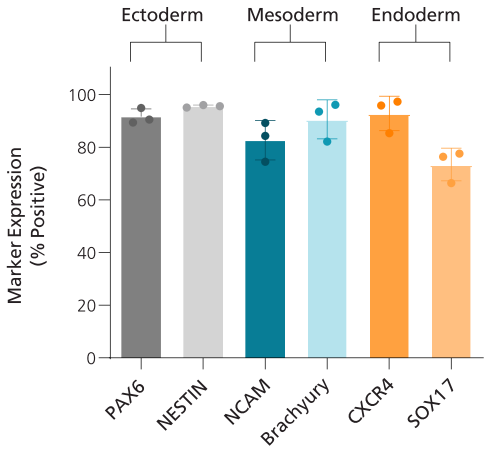


Figure 6. iPSCdirect™ Demonstrates a High Trilineage Differentiation Capacity

iPSCdirect™ cells were split into 3 groups, differentiated using the STEMdiff™ Trilineage Differentiation Kit (Catalog #05230), and subjected to flow cytometry analysis. Expression of PAX6 and Nestin, NCAM and Brachyury (T), and CXCR4 and SOX17 confirm differentiation to the ectoderm, mesoderm, and endoderm lineages, respectively. Bars represent mean marker expression for each group of cells (dots represent the average of 3 technical replicates; error bars represent standard deviation; n = 3 biological replicates). All lineage-specific markers were expressed by more than 70% of differentiated cells.

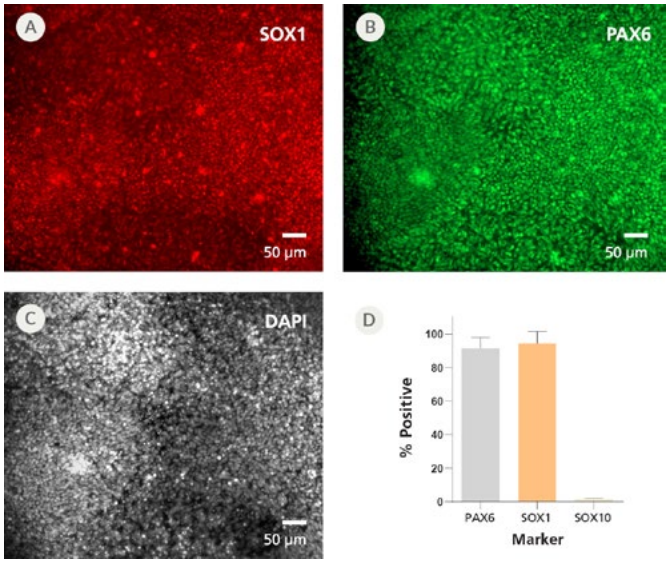


Figure 7. iPSCdirect™ Can Efficiently Differentiate into Neural Progenitor Cells Upon Thawing

Neural progenitor cells (NPCs) were generated from iPSCdirect™ using the protocol in the Product Information Sheet followed by the monolayer protocol from Day 1 in the STEMdiff™ SMADi Neural Induction Kit Technical Manual (Catalog #08581). After 7 days, the resulting NPCs were fixed for immunocytochemistry. They expressed neural progenitor markers (A) SOX1 and (B) PAX6, and nuclei were visualized with (C) DAPI. (D) Marker expression was quantified and negative control SOX10 expression was minimal. Error bars represent standard deviation (n = 2 biological replicates).

Learn more at www.stemcell.com/ipscdirect

Cell Line Quality Checklist

STEMCELL's iPSCs are Developed in Compliance with the ISSCR Standards

The International Society for Stem Cell Research (ISSCR) recently released Standards for Human Stem Cell Use in Research,[1] a set of consensus recommendations that establish minimum characterization and reporting criteria for working with human stem cells that are increasingly adopted in the field, including by journals and granting agencies.

As a passionate advocate for standardizing data reporting and quality control measures, STEMCELL adheres to these Standards in the manufacture of its cell lines to enable the maintenance of high-quality cells for reproducible research. Our quality control process incorporates all of the experiments required to comply with the Standards as outlined below.

ISSCR Standards		SCTi003-A, SCTi004-A	iPSCdirect™
Metadata	Name (or names)/alias of line	✓	✓
	Unique ID/Registry # (name of registry)	✓	✓
	Source (vendor and catalog number if obtained commercially); biopsy site and derivation details (if derived)	✓	✓
	Additional metadata as applicable (e.g. sex, ethnicity, disease information, known mutations, etc.)	✓	✓
Culture Details	Passaging/dissociation/split ratio	✓	N/A – product is single-use
	Freezing and thawing	✓	✓
	Culture reagents used (e.g. media, matrices, growth factors, etc.) with vendor and catalog number	✓	✓
	The passage number of the cryopreserved/characterized master cell bank or working cell bank stocks used, and the number of subsequent passages prior to and during experimentation	✓	✓
Basic Characterization	Authentication	✓	✓
	Mycoplasma	✓	✓
	Sterility (bacteriostasis/fungistasis)	✓	✓
Genomic Characterization	Methodology used including sufficient detail to allow an assessment of sensitivity (e.g. the number of cells analyzed/resolution/depth of analysis)	✓	✓
	Timing of analysis in relation to key experiments reported	User-determined	User-determined
Characterization of Pluripotency and the Undifferentiated State (PSCs only)	Assay methodology	✓	✓
	Quantitative results along with statistical analysis	✓	✓
	Timing of analysis in relation to key experiments reported	User-determined	User-determined
Molecular Characterization	Confirmation of disease mutation (if applicable)	User-determined	User-determined
	Confirmation of genetic modification (if applicable)	User-determined	User-determined
Experimental Details	Information regarding the experimental unit or sample type for each experiment (e.g. individuals, cell lines, clones, tissues, organoids, devices, batches, cells, etc.)	User-determined	User-determined
	Number of replicates (biological/technical)	User-determined	User-determined
Data Practices	Statistical methods used	User-determined	User-determined
	Inclusion of the data and annotation code/software used for phenotype classification for computationally derived classifiers (if applicable)	User-determined	User-determined

For detailed compliance with the ISSCR Standards for your line of interest, please contact your local sales representative.

hPSC-Derived Cells

Human iPSC-Derived Neural Progenitor Cells

Integrate quality into your neural workflow from the start with high-quality, ready-to-use Human iPSC-Derived Neural Progenitor Cells (NPCs; Catalog #200-0620 and #200-0621). These cryopreserved central nervous system (CNS)-type progenitors are differentiated from the robust, extensively tested human induced pluripotent stem cell (iPSC) control line, SCTi003-A (Catalog #200-0511), derived from healthy female donor peripheral blood mononuclear cells (PBMCs). Ready to use directly from thawing, these human NPCs are multipotent, suitable for customized downstream workflows, and compatible with the STEMdiff™ neural system to generate various CNS cell types, such as forebrain neurons, midbrain neurons, and astrocytes. NPCs can be expanded using STEMdiff™ Neural Progenitor Medium (Catalog #05833), allowing for scale-up and reducing the cost of workflows that require large numbers of cells. Cryopreserve expanded NPCs using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838) for flexibility in your experimental schedule.

This research-use-only (RUO) product has been consented for both academic and commercial use. SCTi003-A is derived from cells that are ethically sourced using Institutional Review Board (IRB)-approved consent forms and protocols. These cells are karyotypically stable, demonstrate trilineage differentiation potential, express undifferentiated cell markers, and were reprogrammed using a non-integrating reprogramming technology. Registration with hPSCreg® ensures ethical and biological conformity based on community standards.

Note: For research use or in vitro laboratory-based tissue culture work only. Not approved for application into humans under any circumstances

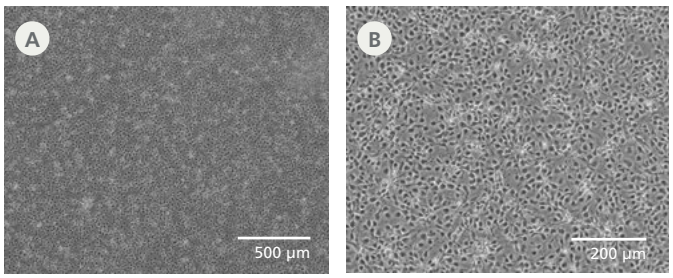


Figure 8. Human iPSC-Derived Neural Progenitor Cells Exhibit High-Quality Morphology Characteristic of Multipotent Central Nervous System Progenitor Cells

Cryopreserved Human iPSC-Derived Neural Progenitor Cells were thawed and plated onto Corning® Matrigel®-coated plates at 200,000 cells/cm^2. NPCs were incubated for 24 hours in STEMdiff™ Neural Progenitor Medium at 37°C and subsequently analyzed by brightfield microscopy. NPCs display the small, teardrop-shaped morphology expected for NPCs. (A) 10X magnification, (B) 20X magnification.

Why Use iPSC-Derived Cells?

- Save time by starting your PSC workflow with highly characterized differentiated cells
- Fully compatible and performance-tested with the STEMdiff™ family of media
- Obtain high-quality differentiated cells, derived from STEMCELL's highly characterized iPSC control lines

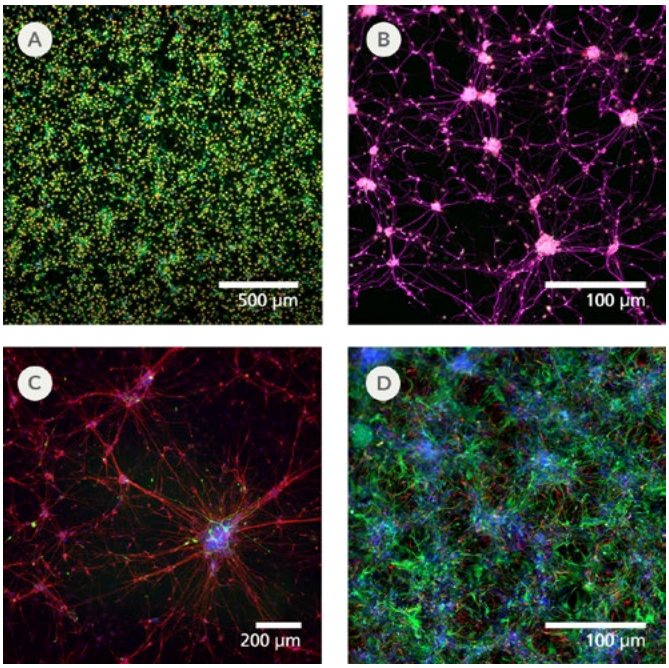


Figure 9. Human iPSC-Derived Neural Progenitor Cells Can Effectively Differentiate into Forebrain Neurons, Midbrain Neurons, and Astrocytes

Human iPSC-Derived Neural Progenitor Cells generated from SCTi003-A iPSCs were thawed, established in culture, and fixed for immunocytochemistry. (A) The NPCs express neural progenitor markers SOX1 (red) and PAX6 (green). (B) NPCs cultured with the STEMdiff™ Forebrain Neuron kit produce forebrain neuron cell populations expressing neuronal identity marker βIII-TUB (magenta). (C) NPCs cultured with the STEMdiff™ Midbrain Neuron kit produce midbrain neuron cell populations expressing neuronal identity marker βIII-TUB (red) and dopaminergic neuron marker TH (green). (D) NPCs cultured with the STEMdiff™ Astrocyte kit produce astrocyte populations expressing astrocyte marker S100β (green) and GFAP (red).

Learn more at www.stemcell.com/NPCs

Stay in the Know

Get notified as soon as new iPSC-derived products are available to order

Reprogramming

ReproRNA™-OKSGM

Generate iPS Cells Using a Non-Integrating Reprogramming Vector

ReproRNA™-OKSGM (Catalog #05931) is a single-stranded RNA replicon vector that contains five reprogramming factors: OCT4, KLF4, SOX2, GLIS1, and c-MYC, as well as a puromycin-resistance gene. This RNA vector reprograms human somatic cells, such as fibroblasts, into induced pluripotent stem (iPS) cells with high efficiency and only requires a single transfection. As shown in Figure 10, using ReproRNA™-OKSGM with ReproTeSR™ (Catalog #05920) reprogramming medium allows for iPS cell colony generation under feeder-free conditions with similar reprogramming efficiency to feeder-based systems. ReproRNA™-derived human iPS cell colonies also express markers of undifferentiated cells and retain a normal karyotype. Subsequently, human iPS cells generated with ReproRNA™-OKSGM can be maintained in a TeSR™ maintenance medium and further differentiated into cells of all three germ layers.

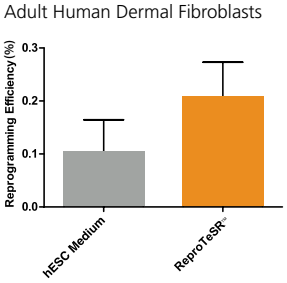


Figure 10. ReproRNA™-OKSGM Vector Efficiently Reprograms Fibroblasts

Human dermal fibroblasts were transfected with the ReproRNA™-OKSGM vector and reprogrammed under feeder-dependent (standard KOSR-containing human embryonic stem cell medium on inactivated mouse embryonic fibroblasts) or feeder-free conditions (ReproTeSR™ on Corning®Matrigel®). The efficiency of reprogramming fibroblasts with ReproRNA™-OKSGM and ReproTeSR™ is comparable to that reported with Sendai virus[3] (n ≥ 6; data shown are mean ± SD).

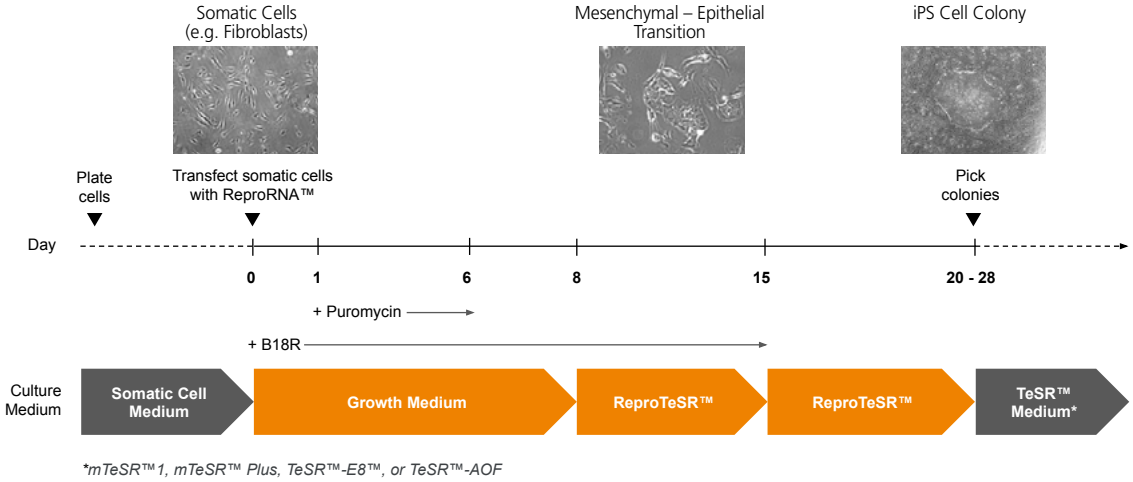


Figure 11. Schematic for Reprogramming with ReproRNA™-OKSGM

Somatic cells are transfected with ReproRNA™-OKSGM at Day 0 and cultured in Growth Medium (containing puromycin). After 5 days of puromycin selection post-transfection, cells are cultured in ReproTeSR™ for the remainder of the reprogramming induction phase until iPS cell colonies emerge. Recombinant B18R Protein is also added during the first 2 weeks after transfection to inhibit the interferon response and increase cell viability. Typically, by Day 20, iPS cell colonies are large enough to be isolated and propagated in a TeSR™ maintenance medium.

Learn more at www.stemcell.com/ReproRNA-OKSGM

ReproTeSR™

Reproducibly Generate High Numbers of Human iPS Cell Colonies

ReproTeSR™ (Catalog #05926) is a complete, defined, xeno-free, and feeder-free reprogramming medium optimized for the generation of human iPS cells. Use ReproTeSR™ during the induction phase of reprogramming to produce more iPS cell colonies than with traditional KOSR-containing human ES cell media. Human iPS cell colonies generated with ReproTeSR™ express undifferentiated cell markers and exhibit more defined borders, compact morphology, and reduced differentiation.

ReproTeSR™ was optimized for reprogramming blood cells and seamlessly integrates with RosetteSep™, SepMate™, EasySep™, and StemSpan™ products for isolating and expanding hematopoietic cells. It can also be used to reprogram other somatic cell types and can be paired with ReproRNA™-OKSGM (Catalog #05931) for reprogramming fibroblasts. iPS cells generated with ReproTeSR™ can be subsequently cultured in a TeSR™ maintenance medium and differentiated with the STEMdiff™ suite of products to cells of all three lineages. Purchase ReproTeSR™ individually or as part of the Erythroid (Catalog #05924) or CD34+ (Catalog #05925) Progenitor Reprogramming Kits.

Integrated Sets of Tools for Reprogramming Human Blood Cells

Erythroid Progenitor Reprogramming Kit

- Enrich cells with RosetteSep™ and SepMate™
- No isolation step required
- Expand erythroid cells with StemSpan™ SFEM II + Erythroid Expansion Supplement
- Reprogram cells with ReproTeSR™

CD34+ Progenitor Reprogramming Kit

- Enrich cells with RosetteSep™ and SepMate™
- Isolate CD34+ cells with EasySep™*
- Expand CD34+ cells with StemSpan™ SFEM II + CD34+ Expansion Supplement
- Reprogram cells with ReproTeSR™

*EasySep™ magnet is not included with the CD34+ Progenitor Reprogramming Kit and must be purchased separately.

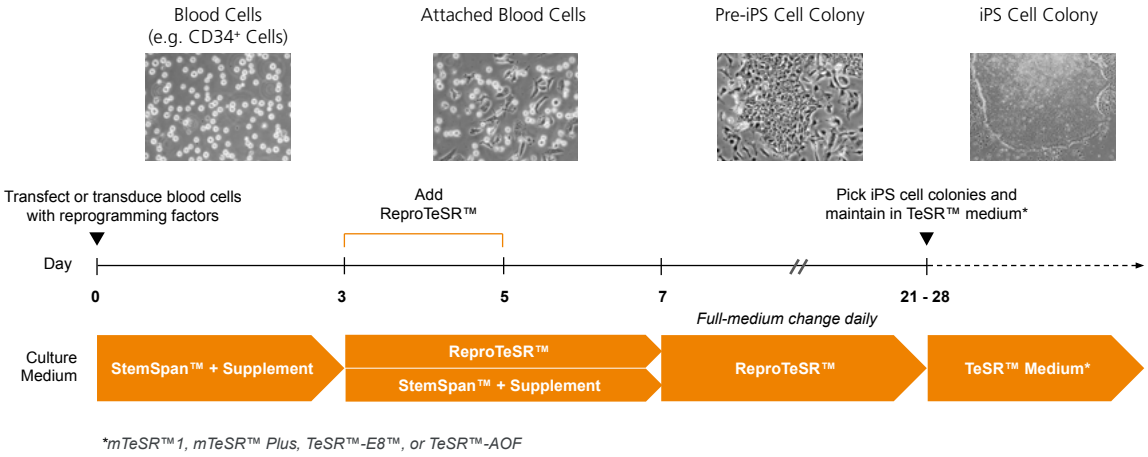


Figure 12. Schematic of ReproTeSR™ Blood Reprogramming Timeline

ReproTeSR™ is used during the entire induction phase of reprogramming (Days 3 to 21). On Days 3 and 5, ReproTeSR™ is added to StemSpan™ growth medium (in a fed-batch manner) to facilitate attachment of transfected cells. Attached cells are further cultured in ReproTeSR™ with daily full-medium changes until putative iPS cell colonies emerge (Days 21 to 28). iPS cell colonies can then be isolated and propagated in a TeSR™ maintenance medium.

Learn more at www.stemcell.com/ReproTeSR

TeSR™-E7™

Generate iPS Cells from Fibroblasts Without Feeders or Animal Components

TeSR™-E7™ (Catalog #05914) is a defined, animal component-free (ACF) reprogramming culture medium optimized for the generation of human iPS cells without the use of feeders. It is based on the E7 formulation published by the laboratory of Dr. James Thomson.[4] TeSR™-E7™ is specifically formulated to limit fibroblast overgrowth, resulting in colonies with easily recognizable ES cell-like morphology.

Why Use TeSR™-E7™?

- Easily identify and select iPSC colonies by using a medium with pre-screened components that ensures high-quality cells
- Rapidly establish homogeneous iPSC cultures with reduced differentiation and fibroblast growth
- Enjoy reproducibly efficient human iPSC generation with a feeder-free, defined formulation

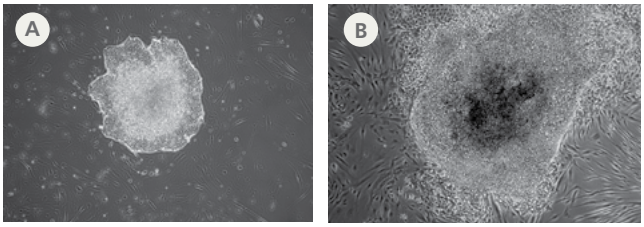


Figure 13. Primary iPS Cell Colonies Derived in TeSR™-E7™ Have Defined Borders and Reduced Differentiation

(A) TeSR™-E7™ yields easily recognizable iPS cell colonies with defined borders. (B) Unqualified components can result in colonies that have poorly defined edges and higher levels of differentiation. Representative colonies from adult human fibroblasts reprogrammed with episomal vectors containing OCT4, SOX2, KLF4, and c-MYC are shown.

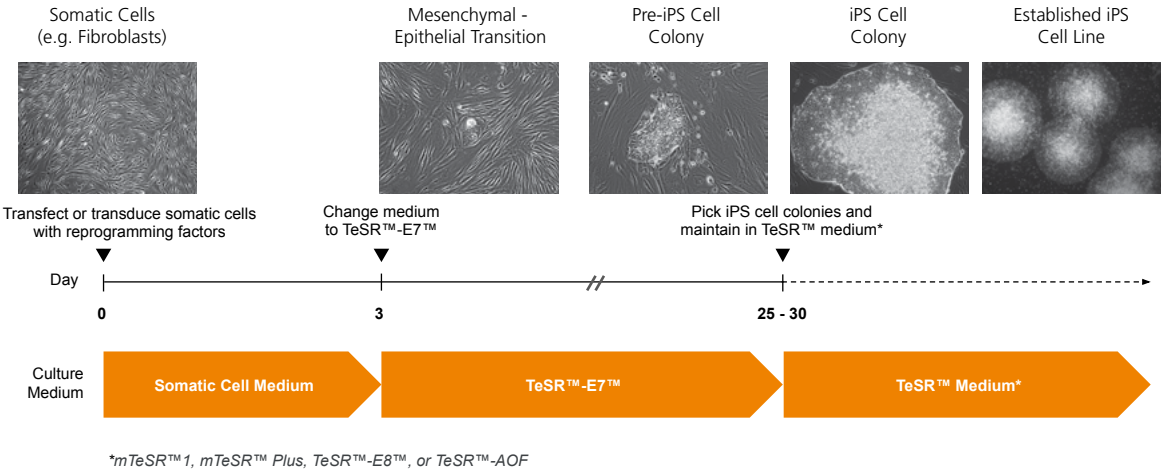


Figure 14. Schematic of Reprogramming Timeline

TeSR™-E7™ can be used during the entire induction phase of reprogramming (Days 3 to 25+). Following reprogramming, iPS cell colonies can be isolated and propagated in feeder-free maintenance systems (e.g. TeSR™ media on Corning® Matrigel® or Vitronectin XF™ matrices). TeSR™ = TeSR™ family of maintenance media.

Learn more at www.stemcell.com/TeSR-E7

Cryopreservation

Increase Post-Thaw Recovery and Viability with Serum-Free Cryopreservation Media

mFreSR™ and FreSR™-S

Conventional methods for cryopreservation of human pluripotent stem cells (hPSCs) use fetal bovine serum, introducing an undefined component into the culture system. FreSR™ cryopreservation media are defined, serum-free, and optimized for use with cells cultured with TeSR™ maintenance media. Cells stored in FreSR™ media have higher recovery and maintain higher viability post-thaw than cells frozen via conventional methods using serum.[5-8] mFreSR™ (Catalog #05855) serum-free medium is optimized for cryopreservation of hPSCs as aggregates. FreSR™-S (Catalog #05859) animal component-free media is optimized for cryopreservation of cells in single-cell suspension and provides faster post-thaw recovery of hPSC cultures compared with conventional freezing methods.

Why Use mFreSR™ and FreSR™-S?

- Obtain more cells with high post-thaw viability and recovery
- Enjoy seamless compatibility with hPSCs cultured in TeSR™ media
- Reduce viral contamination risk with animal component-free FreSR™-S, optimized for single-cell suspensions

Learn more at www.stemcell.com/mFreSR

Learn more at www.stemcell.com/FreSR-S

CryoStor® CS10

Storage and cryopreservation of cells and tissues are important parts of the workflow for biological research. CryoStor® CS10 (Catalog #07930) is animal component-free, cGMP-manufactured with USP grade components, and designed to maintain high viability and maximize hPSC cell recovery after long-term storage. CryoStor® CS10 contains 10% dimethyl sulfoxide (DMSO) and provides a safe and protective environment for cells and tissues during the freezing, storage, and thawing processes.

Learn more at www.stemcell.com/CryoStor-CS10

ThawSTAR® Automated Thawing Systems

Standardize Your Thawing Performance Through Automation

Increase confidence in your cell thawing workflow with consistent thawing performance and sample sterility by using the ThawSTAR® CFT2 (Catalog #100-0650) and ThawSTAR® CB (Catalog #100-1151) Automated Thawing Systems. With a standardized thawing process that replaces manual, water bath-based thawing, ThawSTAR® systems eliminate the risk of contamination and deliver controlled thawing profiles. Utilize ThawSTAR® CFT2 and ThawSTAR® CB to easily and consistently thaw cryogenic vials and cryobags, respectively. Simply insert a frozen sample and retrieve it once the device alerts you at the end of the thaw cycle.

Utilize the ThawSTAR® CFT2 Confirmation Vials (Catalog #100-0643), ThawSTAR® CFT2 Transporter (Catalog #100-0642), and ThawSTAR® CB Barrier Bags (Catalog #100-1153) within your workflow to document instrument performance, handle and transport frozen vials, and ensure proper positioning during thawing, respectively. Facilitate functional testing of the ThawSTAR® systems using the ThawSTAR® CFT2 IOPQ (Catalog #100-0730) and ThawSTAR® CB IOPQ (Catalog #100-1152) Kits, which include installation, operational, and performance qualification documentation and accessories.

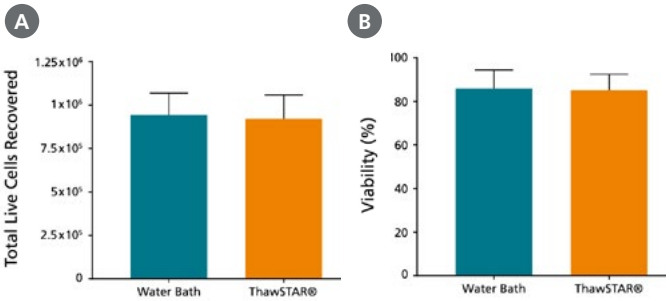


Figure 15. Frozen hPSCs Thawed Using the ThawSTAR® CFT2 Automated Thawing System Show High Recovery and Viability

hPSCs cryopreserved in CryoStor® CS10 at a concentration of 1 x 10^6 cells/vial were retrieved from liquid nitrogen one week after storage. When thawed using the ThawSTAR® CFT2 Automated Thawing System or a water bath, (A) the mean live cell recovery was 9.05 x 10^5 vs. 9.35 x 10^5 cells, respectively, and (B) the mean viability was 83.04% vs. 82.93%, respectively. The hPSCs were from 3 different cell lines (M001, 1C, and H9) and were tested in triplicates. Cell recovery and viability was assessed using a Nucleoview™ counter.

Learn more at www.stemcell.com/thawstar

Tech Tip
Cryopreservation and Thawing of Pluripotent Stem Cells

Characterization

hPSC Genetic Analysis Kit

Detect Most Karyotypic Abnormalities in Human ES and iPS Cells Using qPCR

The hPSC Genetic Analysis Kit (Catalog #07550) contains primer/probe mixes to detect the majority of karyotypic abnormalities reported in human embryonic stem (ES) and induced pluripotent stem (iPS) cells. This qPCR-based kit enables the genetic screening of multiple human ES and iPS cell lines in a rapid and cost-effective manner, and includes enough material to analyze 20 individual samples in triplicate. Our online hPSC Genetic Analysis Tool (**www.stemcell.com/geneticanalysisapp**) is designed to help with data analysis and interpretation: simply input qPCR data and the tool will perform statistical analyses, assist with data interpretation, and provide visual representation of the data.

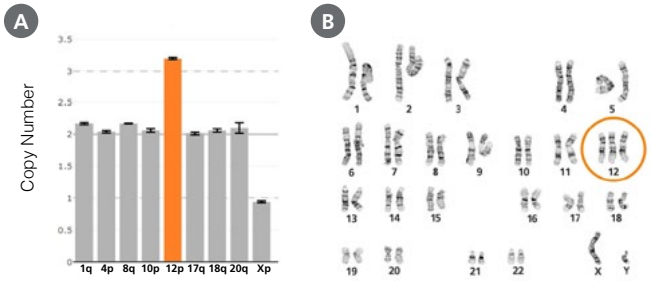


Figure 16. The hPSC Genetic Analysis Kit Identifies Chromosome 12 Trisomy

Chromosome 12 trisomy in the WLS-1C human iPS cell line is (A) detected using the hPSC Genetic Analysis Kit and (B) confirmed by G-banding.

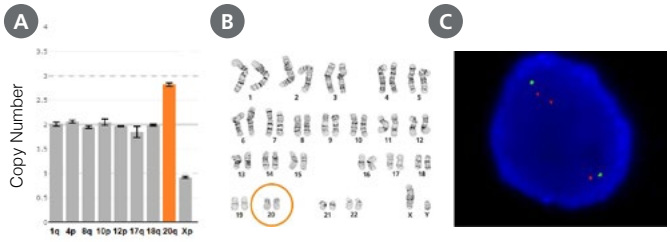


Figure 17. The hPSC Genetic Analysis Kit Identifies Chromosome 20q11.21 Duplication

Chromosome 20q duplication in the WLS-1C human iPS cell line is (A) detected using the hPSC Genetic Analysis Kit, (B) undetected by G-banding, and (C) confirmed by fluorescent in situ hybridization using probes for 20p11 (green) and 20q11.21 (red).

Video

How to Set Up an Assay with the hPSC Genetic Analysis Kit

Learn more at www.stemcell.com/GeneticAnalysisKit

Why Use the hPSC Genetic Analysis Kit?

- Detect the most common karyotypic abnormalities observed in hPSC cultures
- Generate results within one day
- Enable more frequent screening with this cost-effective kit
- Interpret results easily with the online hPSC Genetic Analysis Tool

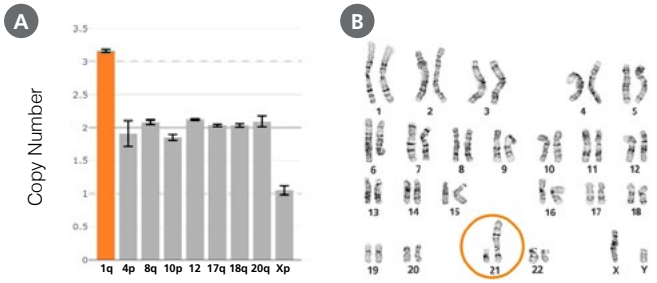


Figure 18. The hPSC Genetic Analysis Kit Identifies Chromosome 1 Duplication via Unbalanced Translocation

Unbalanced rearrangement of chromosome 1 in the WLS-1C human iPS cell line, in which an extra copy of the long (q) arm of chromosome 1 translocated to the short arm (p) of chromosome 21, was (A) detected using the hPSC Genetic Analysis Kit and (B) confirmed by G-banding.

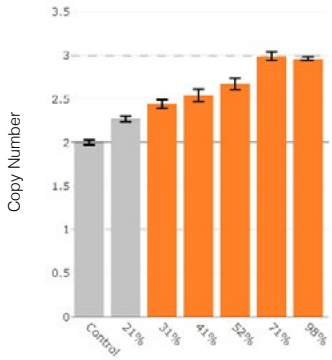


Figure 19. The hPSC Genetic Analysis Kit Identifies Abnormalities in Cultures with Approximately 30% Mosaicism

Genetically normal WLS-1C human iPS cells were mixed in the indicated ratios with WLS-1C human iPS cells containing a chromosome 20q duplication. Cultures with approximately 30% genetically abnormal cells exhibit a significantly enriched population of 20q11.21 duplication (orange bars).

STEMdiff™ Trilineage Differentiation Kit

Validate Pluripotency with Directed Differentiation

The STEMdiff™ Trilineage Differentiation Kit (Catalog #05230) provides a simple cell culture assay to functionally and reproducibly validate the ability of human ES and iPS cells to differentiate to the three germ layers. This kit includes reagents and protocols to perform parallel in vitro directed differentiation experiments for each germ layer, clearly establishing trilineage differentiation potential within one week. Clear, quantitative assay results evaluated by immunocytochemistry, flow cytometry, or transcriptome analysis make the STEMdiff™ Trilineage Differentiation Kit a valuable tool for establishing the pluripotency of human ES and iPS cell lines.

Figure 20. Molecular Analysis of Cultures Differentiated with the STEMdiff™ Trilineage Differentiation Kit Shows Strong Separation of Lineage-Specific Markers

H9 cells were maintained in mTeSR™1 and subsequently differentitated in vitro using either directed differentiation with the STEMdiff™ Trilineage Differentiation Kit or spontaneous differentiation in embryoid bodies (EBs) using a 10-day protocol in serum-containing medium. Undifferentiated cells, differentiated ectoderm, mesoderm, and endoderm cells from the directed differentiation kit and EBs were then subjected to a microarray-based transcriptome analysis to evaluate expression levels of key germ layer markers. Cells differentiated using the STEMdiff™ Trilineage Differentiation Kit showed clear upregulation of appropriate germ layer-specific markers, whereas the same cells differentiated spontaneously in EBs did not show significant upregulation of mesoderm or endoderm markers.

Learn more at www.stemcell.com/TrilineageKit

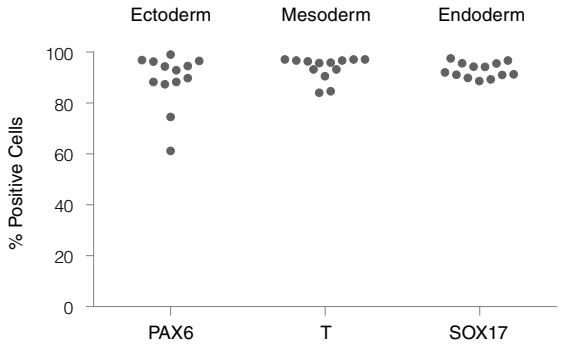


Figure 21. The STEMdiff™ Trilineage Differentiation Kit Promotes Efficient Differentiation to All Three Germ Layers

Pluripotent stem cells (both iPS and ES cells represented) were maintained in mTeSR™1, differentiated using the STEMdiff™ Trilineage Differentiation Kit, and subjected to flow cytometry analysis (n = 13 biological replicates, including 5 distinct cell lines). The markers used for flow cytometry for each germ layer are listed below the x-axis.

hPSC Trilineage Differentiation qPCR Array

The hPSC Trilineage Differentiation qPCR Array (Catalog #07515) provides a validated 90-gene assay to assess gene expression associated with undifferentiated hPSCs or their derivatives undergoing the early stages of differentiation, plus housekeeping controls and a synthetic DNA positive control. Data analysis is streamlined with our flexible online app (**www.stemcell.com/qPCRanalysis**).

Learn more at www.stemcell.com/trilineage-array

Tech Tip

Assessing Differentiation to Three Germ Lineages

Antibodies

For Human Pluripotent Stem Cells and Differentiated Cells

Be confident in your experimental results, save valuable research time, and ensure experimental reproducibility by choosing antibodies from STEMCELL Technologies. Our high-quality primary and secondary antibodies are verified to work with our pluripotent stem cell reagents in specific applications, ensuring that your downstream cell analysis, including phenotyping and purity assessments, works consistently.

Popular Antibodies for hPSC Research

Target Antigen	Clone	Isotype	Catalog #
OCT4 (OCT3)	3A2A20	Mouse IgG2b	60093
OCT4 (OCT3)	40	Mouse IgG1	60059
SSEA-1 (CD15)	MC-480	Mouse IgM	60060
SSEA-3	MC-631	Rat IgM	60061
SSEA-4	MC-813-70	Mouse IgG3	60062
SSEA-5	8e11	Mouse IgG1	60063
TRA-1-60	TRA-1-60R	Mouse IgM	60064
TRA-1-81	TRA-1-81	Mouse IgM	60065
TRA-2-49	TRA-2-49/6E	Mouse IgG1	60066
TRA-2-54	TRA-2-54/2J	Mouse IgG1	60067

For a complete listing of antibodies and conjugates available, visit **www.stemcell.com/antibodies**.

GloCell™ Fixable Viability Dyes

For Live/Dead Cell Staining

GloCell™ Fixable Viability Dyes are fluorescent amine-labeling dyes for live/dead staining of mammalian cells, allowing clear exclusion of dead cells from flow cytometry data. These dyes are resistant to washing and fixation and are compatible with intracellular antibody staining protocols. Stained cells can also be cryopreserved without loss of fluorescence intensity.

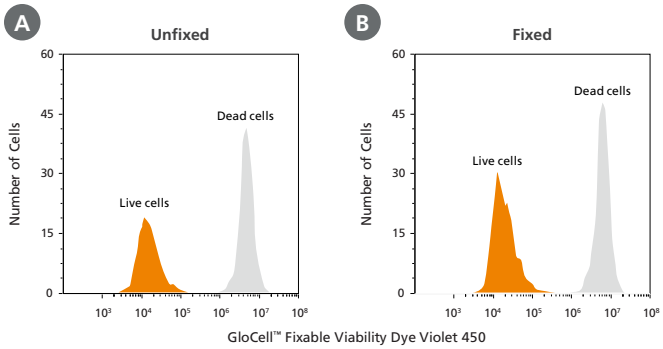


Figure 22. Fluorescence Signals in Unfixed and Fixed Cells Stained with GloCell™ Fixable Viability Dye Violet 450 Are Preserved

A mixture of live and dead (heat-shocked at 95°C for 30 minutes) WLS-1C human iPS cells were stained with GloCell™ Fixable Viability Dye Violet 450 (Catalog #75009) with or without fixation in 4% paraformaldehyde. After staining, (A) unfixed and (B) fixed cells were immediately analyzed by flow cytometry.

Learn more at www.stemcell.com/GloCell

Annexin V Dyes & Caspase 3/7 Assay Reagents

For Detection of Early-Stage Cell Apoptosis

Annexin V is a characteristic cell death marker that can be used to specifically detect early apoptotic mammalian cells. The Annexin V Apoptosis Detection Kit can be used for the combined detection of early-stage cell apoptosis using Annexin V and late-stage cell apoptosis or necrosis using both Annexin V and 7-Aminoactinomycin D (7-AAD).

Caspase 3/7 is widely accepted as a reliable indicator of apoptosis, since caspase 3 activation is a necessary step to initiate the apoptotic cascade in a broad spectrum of cell types.

STEMCELL's caspase 3/7 products can be used to detect caspase 3/7 activity in apoptotic cells, are robust in detecting caspase 3/7 activity, and can be easily adapted to be used as high throughput assays for flow cytometry and microplates.

Maintenance and Expansion: Maintenance Media

Select the Right Maintenance Medium for Your Research

Maintaining high-quality human pluripotent stem cells (hPSCs), including induced pluripotent stem (iPS) and embryonic stem (ES) cells, is critical for success in hPSC research. The TeSR™ family of feeder-free maintenance media is manufactured using rigorously pre-screened materials to ensure the highest levels of batch-to-batch consistency and experimental reproducibility. These media are based on published formulations[9-10] from the laboratory of Dr. James Thomson and are tailored to suit your specific needs.

cGMP Media

Minimize Risk in Your Cell Therapy Development

TeSR™-AOF

- Contains no animal-derived raw materials to the secondary level of manufacturing
- Supports high-quality culture morphology, robust attachment, and cell expansion
- Stabilized FGF2 supports high cell quality while allowing for alternate feeding schedules

Versatility for Routine Maintenance and Expansion

mTeSR™ Plus

- Allows for alternate feeding schedules due to enhanced pH buffering and stabilization of FGF2
- Improves upon the trusted mTeSR™1 formulation to provide superior culture morphology as well as cell growth and survival rates
- Maintains cell quality, from iPSC line manufacturing, to preparing hPSCs for downstream differentiation with our STEMdiff™ kits

mTeSR™1

- Used to maintain thousands of hPSC lines in over 60 countries for more than 15 years
- Contains pre-screened BSA to stabilize medium, aid in lipid/nutrient transport, and protect cultures from toxins and stresses[4]

Why Use the TeSR™ Media Family?

- Minimize variability by choosing feeder-free formulations to limit the presence of undefined components
- Maintain and passage hPSCs with confidence by using the most widely published media family for hPSC culture, with >9000 peer-reviewed publications
- Ensure cell quality by using the TeSR™ medium that best suits your research needs, from clonal selection to scaling up in 3D suspension cultures

Overview of TeSR™ Maintenance Media
www.stemcell.com/TeSR

Specialized Media

Enhance Single-Cell Passaging

NEW: eTeSR™

- Enhanced buffering, stabilization of FGF2, and optimized metabolites support superior culture morphology and cell growth while allowing for alternate feeding schedules
- Increases yields while reducing stress associated with single-cell passaging or high density cultures during routine maintenance
- Enables easy transition between media for intermediate single-cell culture steps such as cloning or gene editing

Just the Basics

TeSR™-E8™

- Contains only the 8 most critical components required for hPSC maintenance[4,11]
- Animal component-free (ACF), with no animal-derived raw materials to the primary level of manufacturing
- Has a low-protein formulation, compared to other TeSR™ maintenance media

Suspension Culture

Scale Up Cell Production with Fed-Batch Media

TeSR™-AOF 3D

- Contains no animal-derived raw materials to the secondary level of manufacturing
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 10^9 high-quality hPSCs rapidly without requiring adaptation from 2D culture

mTeSR™3D

- Based on mTeSR™1, optimized for hPSC scale-up
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 10^9 high-quality, undifferentiated hPSCs in as few as 2 - 3 weeks

TeSR™-E8™3D

- Based on TeSR™-E8™, optimized for hPSC scale-up in low-protein, ACF conditions
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 10^9 high-quality, undifferentiated hPSCs in as few as 2 - 3 weeks

Achieve Cell Quality No Matter Your Passaging Method

Use eTeSR™ and mTeSR™ Plus to Support Your Workflow Needs

Maintaining high-quality hPSCs is critical for success in downstream research. At STEMCELL Technologies, our scientists have long recommended routinely passaging hPSCs as aggregates as this method allows for the long-term expansion of many different cell lines while maintaining an expected karyotype. Existing TeSR™ media, including mTeSR™ Plus, were developed using aggregate passaging methods. In some cases, researchers may prefer single-cell passaging for obtaining higher-density cultures, or for compatibility with single-cell applications. For these researchers, we developed eTeSR™, a novel hPSC maintenance medium formulated specifically to maintain cell quality when passaging and maintaining hPSCs as single cells. With eTeSR™ and mTeSR™ Plus, you can take charge of your cell quality in every step of your workflow—regardless of your passaging method.

eTeSR™

Enhanced Maintenance Medium Optimized for Single-Cell Passaging

eTeSR™ (Catalog #100-1215) is an enhanced feeder-free cell culture medium, stabilized and optimized to support the maintenance and expansion of hPSCs when cultured as single cells. The formulation has been developed to reduce cellular stress associated with single-cell passaging and can be used for routine maintenance or application-specific single-cell culture. eTeSR™ builds upon previous TeSR™ formulations[9-10], the most widely published feeder-free cell culture media family for hPSCs.

eTeSR™ has been specifically developed to support single-cell passaging that typically involves shorter passaging schedules and high densities. To cope with the increased metabolic demand and increased cellular stress associated with this method, eTeSR™ is formulated to stabilize key components, including FGF2, improve buffering capacity, and optimize metabolites, to produce high-quality hPSCs with improved genetic stability compared to other hPSC maintenance media.

eTeSR™ is compatible with both daily and restricted feeding schedules while maintaining high cell quality and equivalent performance, and can also be used with a variety of cell culture matrices, including Corning® Matrigel® hESC-Qualified Matrix and CellAdhere™ Laminin-521 (Catalog #77003).

Each lot of eTeSR™ 10X Supplement is quality-tested in a culture assay using hPSCs.

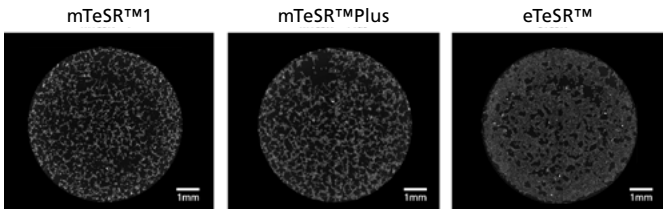


Figure 23. eTeSR™ Improves Attachment Efficiency of hPSCs Seeded As Single Cells

Representative Hoechst staining from the STiPS-R038 hPSC line 24 hours post-seeding. Cells were seeded at 4.7x10⁴ cells/cm² in either mTeSR™1, mTeSR™ Plus, or eTeSR™ supplemented with 10 µM Y-27632 on Matrigel®-coated 96-well plates. Plates were fixed, stained for Hoechst 33342, and imaged using the ImageXpress® Micro 4 Microscope. Scale bars = 1 mm.

Why Use eTeSR™?

- Support cell quality while allowing for alternative feeding schedules with enhanced buffering, stabilization of key components (including FGF2), and optimized metabolites
- Increase cell yields while reducing stress associated with single-cell passaging or high density cultures during routine maintenance
- Complete your workflow with compatible gene editing, cloning, differentiation, and cryopreservation protocols
- Eliminate spontaneous differentiation

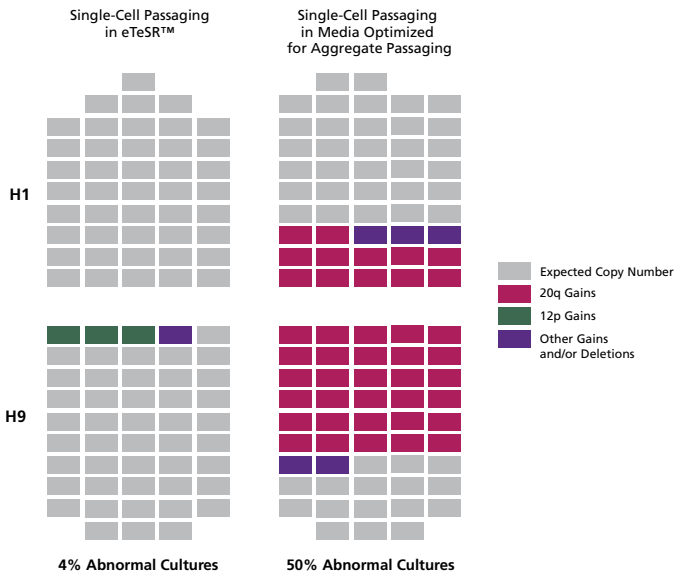


Figure 24. eTeSR™ Demonstrates Improved Genetic Stability of hPSC Cultures When Maintained Long-Term Using Single-Cell Passaging

A significant reduction in the number of genetic variants was detected in individually cloned H1 and H9 hPSC biological replicates when cultured in eTeSR™ compared to media primarily optimized for aggregate passaging as measured by the hPSC Genetic Analysis Kit and confirmed by FISH. Each box/square represents an individual clone cultured for 20 weeks (30 passages).

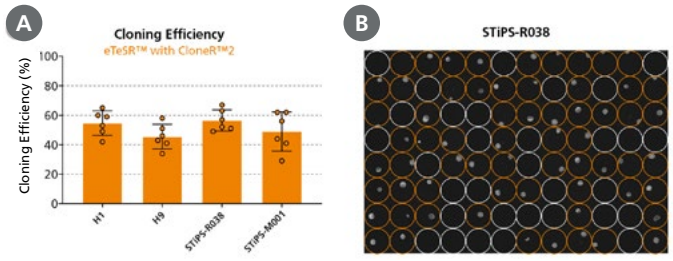


Figure 25. High Cloning Efficiencies Are Achieved Following Single-Cell Deposition Using eTeSR™ with CloneR™2

(A) Four hPSC lines were seeded at 1 cell/well in eTeSR™ supplemented with CloneR™2 on Vitronectin-XF™-coated 96-well plates. Each data point represents one 96-well plate over three independent experiments. The average cloning efficiency across all four cell lines in eTeSR™ was 51 ± 3%. (B) Representative 96-well plate imaged at Day 8 post-plating, showing colonies generated in eTeSR™ using single-cell deposition. Orange circles highlight wells with a colony present.

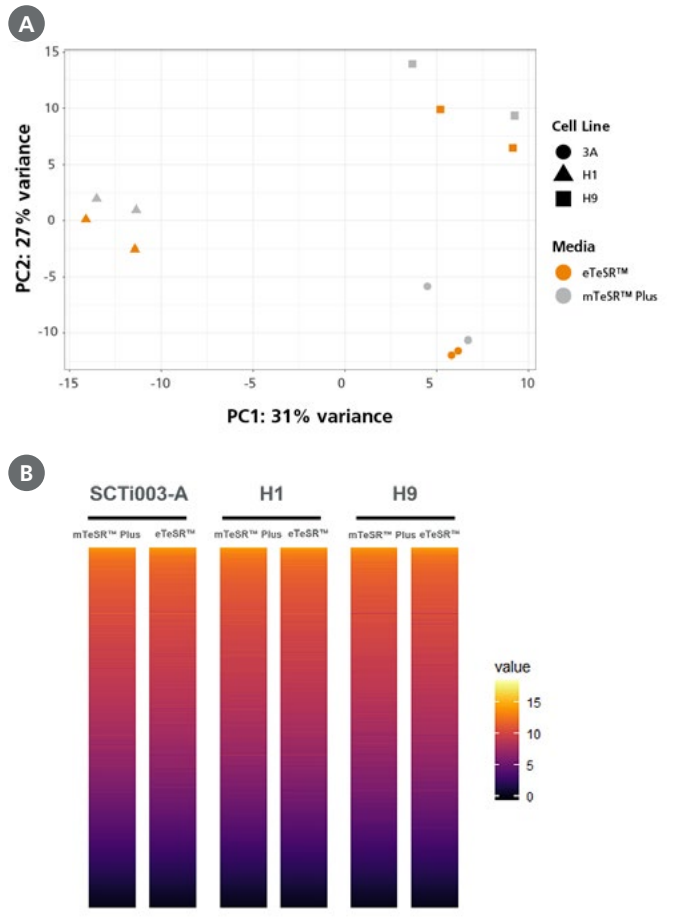


Figure 26. Global Gene Expression Profiles Are Comparable Between eTeSR™ Single-Cell Passaged Cultures and mTeSR™ Plus Aggregate Passaged Cultures

Whole transcriptome analysis was performed using the Illumina NextSeq 500 on three hPSC lines passaged as either aggregates in mTeSR™ Plus or as single cells in eTeSR™. (A) PCA analysis shows that samples cluster by cell line with minimal effect from cell culture medium and passaging technique. (B) A heat map of global gene expression showing comparable gene expression between conditions. No gene ontology or signaling pathway enrichment was detected (n = 3 hPSC lines).

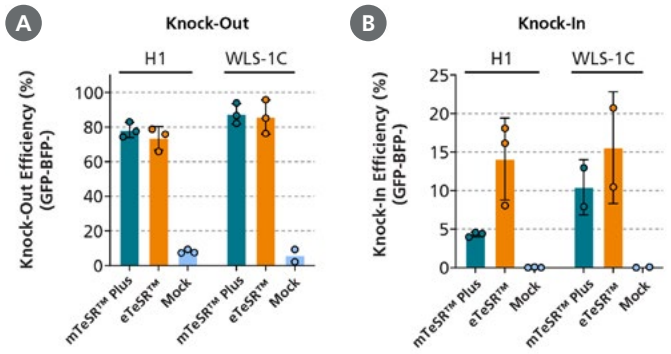


Figure 27. eTeSR™ Supports Efficient Gene-Editing in hPSCs

Using the ArciTect™ CRISPR-Cas9 system, GFP-labeled hPSC lines (H1 and WLS-1C) were electroporated in the presence of a ribonucleic protein (RNP) complex consisting of Cas9 and a guide RNA targeting the GFP sequence. A donor template was also included encoding a two base pair change, resulting in the conversion of the GFP sequence to a BFP sequence, which can be determined using flow cytometry. Data points in the graph represent the gene-editing outcomes of three independent experiments. Both knock-out (A) and knock-in (B) conditions show effective gene-editing with eTeSR™-maintained cultures, displaying a higher knock-in efficiency compared to mTeSR™ Plus-maintained cultures. The mock condition shows eTeSR™-maintained cells that have been incubated with the RNP complex and donor template but have not been electroporated. Error bars represent standard deviation of replicates.

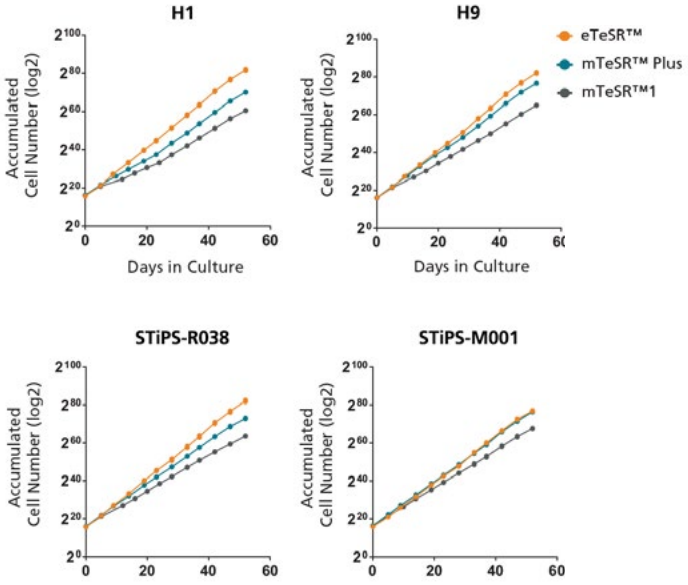


Figure 28. hPSCs Cultured As Single Cells Show Greater Cell Expansion in eTeSR™

Four hPSC lines were single-cell passaged using TrypLE™ and maintained in either mTeSR™1, mTeSR™ Plus, or eTeSR™ on Corning® Matrigel®-coated plates. Cultures were maintained for 11 passages, using daily feeding for mTeSR™1 or a Day 4 and subsequent Day 5 restricted feeding schedule for mTeSR™ Plus and eTeSR™. Accumulated cell numbers were calculated by dividing the number of cells at the end of each passage by the number of cells seeded at passage.

Learn more at www.stemcell.com/eTeSR

mTeSR™ Plus

Enjoy Flexible Feeding Schedules and Enhanced Growth Characteristics with Stabilized, cGMP-Grade Medium

mTeSR™ Plus (Catalog #100-0276) is based on the formula of mTeSR™1, the most-published feeder-free hPSC maintenance medium[9-10], and allows for culture versatility that supports high-quality maintenance and expansion of hPSCs.

To enhance cell quality attributes, critical medium components—including FGF2—have been stabilized, while medium pH is more consistent due to enhanced buffering. As a result, this medium supports higher cell numbers with daily feeding, while maintaining consistent quality during restricted feeding schedules.

mTeSR™ Plus is manufactured under relevant cGMPs. With enhanced critical raw material traceability, processes, and quality control validations, mTeSR™ Plus enables a seamless transition from basic research to drug and cell therapy development.

mTeSR™ Plus is compatible with a variety of culture matrices, including Corning® Matrigel® hESC-Qualified Matrix, CellAdhere™ Laminin-521 (Catalog #77003), and Vitronectin XF™ (Catalog #07180).

Each lot of mTeSR™ Plus 5X Supplement is used to prepare complete mTeSR™ Plus medium and is then performance-tested in a culture assay using hPSCs.

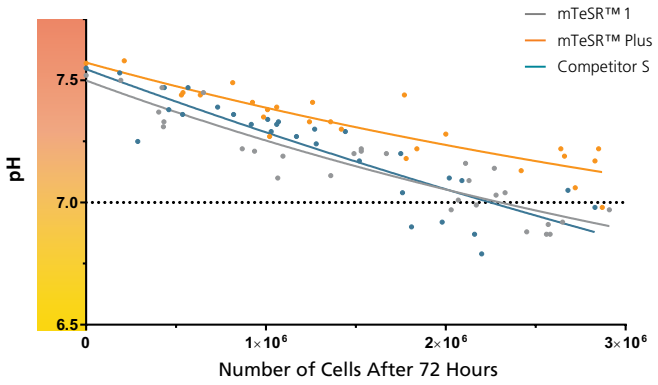


Figure 29. mTeSR™ Plus Maintains Optimal pH Levels Throughout a Weekend-Free Protocol

The pH of spent medium from hPSCs cultured in mTeSR™ Plus is higher than that of hPSCs cultured in mTeSR™1 and another flexible-feeding medium at similar cell densities. pH and cell numbers were measured after a 72-hour period without feeding. The range of cell numbers shown represent different densities that would be observed throughout a typical passage. Cell numbers are from one well of a 6-well plate.

Why Use mTeSR™ Plus?

- Enjoy weekend-free feeding while supporting cell quality, with improved buffering and stabilization of key components
- Achieve superior culture morphology and cell growth characteristics in your aggregate-based cultures
- Complete your workflow with compatible gene editing and differentiation protocols
- Ensure cell safety with a viral-safe medium manufactured under cGMPs

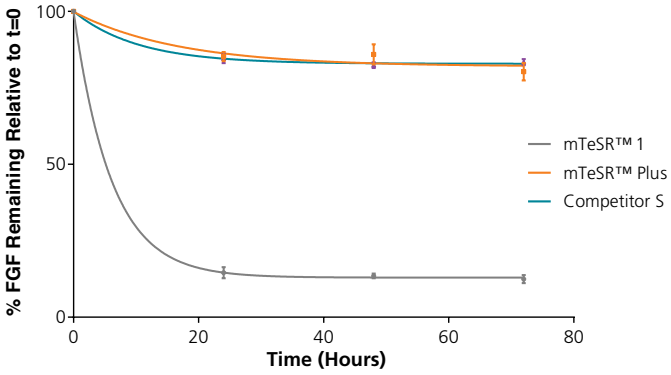


Figure 30. mTeSR™ Plus Maintains Consistent Levels of FGF2 Throughout a Weekend-Free Protocol

FGF2 levels remain high in mTeSR™ Plus when kept at 37°C over a 72-hour time period. Measured by ELISA.

Maintain hPSCs on Your Own Schedule

☑ **Skip 2 days = Double feed**

☑ **Skip 1 day = Regular feed**

The possibilities are endless. Use your regular schedule, or try something new to free up your days.

Passaging Frequency	Mon	Tue	Wed	Thu	Fri	Sat	Sun	
7d	P	F	F	F	F	F	F	repeat
7d	P	F	F	F	2F	X	X	repeat
6d	P	X	2F	X	X	F	repeat	
5d	P	F	2F	X	X	repeat		
3d/4d	P	F	X	P	2F	X	X	repeat
Fill Out Your Own								

P = Passage; F = Single Feed; 2F = Double Feed

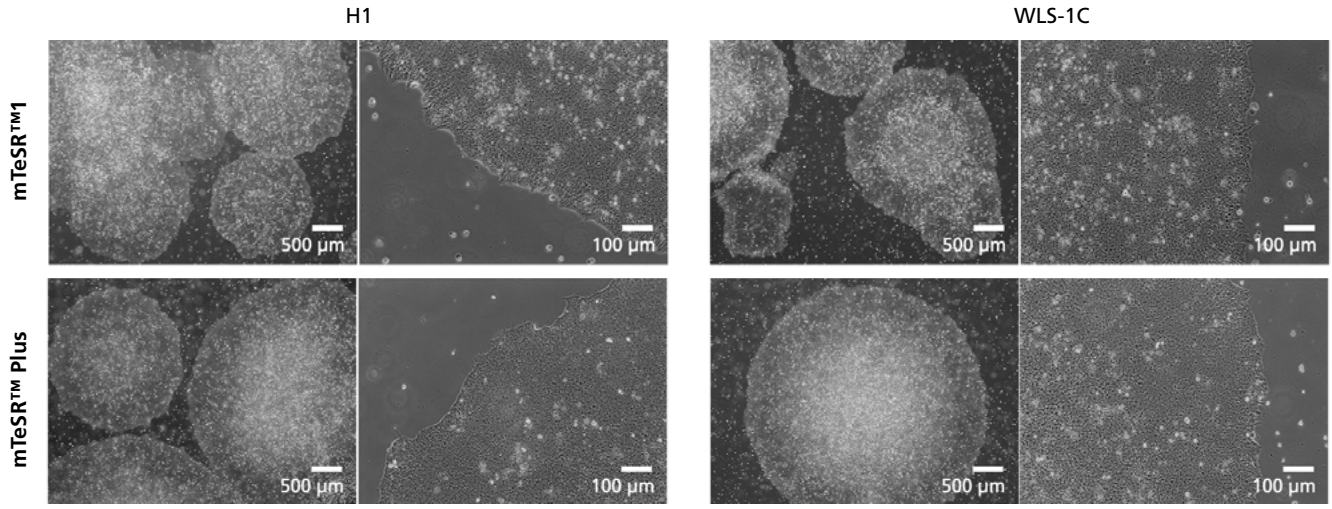


Figure 31. Normal Human ES and iPS Cell Morphology Is Observed in mTeSR™ Plus Cultures

Images depict undifferentiated human ES (H1) and iPS (WLS-1C) cells cultured on Corning® Matrigel® matrix in mTeSR™1 with daily feeds or in mTeSR™ Plus with restricted feeds. Cells retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type after 10 passages. Densely packed cells and multi-layering are prominent when cells are ready to be passaged.

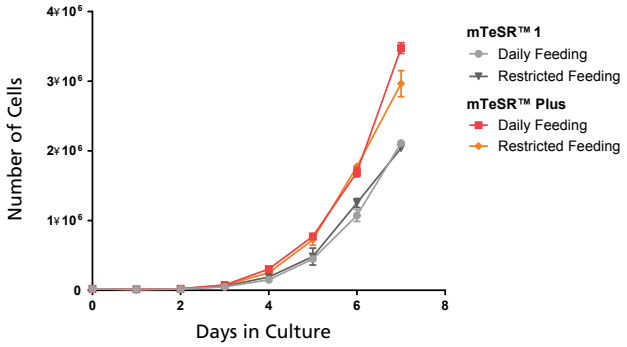


Figure 32. mTeSR™ Plus Supports Higher Cell Numbers, Even with Restricted Feeding

Growth curves were obtained for human ES (H9) cells cultured in mTeSR™1 or mTeSR™ Plus on Corning® Matrigel® matrix over 7 days with either daily feeds or restricted feeds. Growth curves were determined by seeding 20,000 cells per well of a 6-well plate as aggregates and counting the cell numbers each day in duplicate wells.

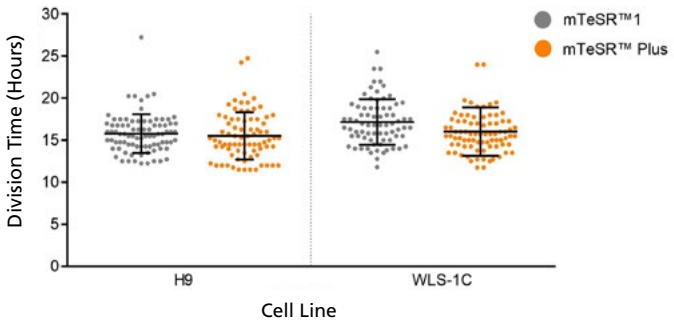


Figure 33. hPSCs Maintained in mTeSR™ Plus Demonstrate Equivalent Cell Division Times to Cells Maintained in mTeSR™1

Human ES and iPS cells (H9 and WLS-1C) cultured in mTeSR™1 or mTeSR™ Plus were dissociated to single cells and seeded at 20,000 cells/cm^2 on Matrigel®-coated plates. The cells were imaged every 20 minutes on an IncuCyte ZOOM® for three days with a medium exchange the day after seeding. Individual cell division times were determined using single cell tracking. Data points include first, second, and third cell divisions.

Learn more at www.stemcell.com/mTeSRPlus

Minimize Risk in Your Cell Therapy Development

Our Commitment to Quality and Good Manufacturing Practices

Choosing a cell and tissue culture medium is a critical step in ensuring the quality and performance of your cultures. For cell therapy manufacturers, this choice can have additional impacts on the safety of your cell therapy. Minimize risk by using animal origin-free TeSR™-AOF as part of a high-compliance maintenance workflow that also includes dissociation reagents (Gentle Cell Dissociation Reagent, ReLeSR™), matrices (CellAdhere™ Laminin-521), and cryopreservation reagents (CryoStor® CS10). For more information on how we can support your regulatory needs, including navigating requirements for using TeSR™-AOF in your cell therapy applications, visit **www.stemcell.com/regulatory-support** or contact your STEMCELL representative.

TeSR™-AOF

Maintain hPSCs for Cell Therapy Development Using cGMP-Grade, Animal Origin-Free, Stabilized Media

Reduce risk and obtain greater numbers of higher quality cells for your human pluripotent stem cell (hPSC)-derived cell therapy development with TeSR™-AOF (Catalog #100-0401), manufactured under relevant cGMPs.

With no raw materials of animal or human origin to the secondary level of manufacturing, enjoy more straightforward traceability and enhanced viral safety compared to media that are only animal origin-free to the primary level of manufacturing.

Use TeSR™-AOF to consistently culture viral-safe, high-quality hPSCs on a schedule that works for you—with whatever cell lines you choose.

To enhance cell quality attributes, particularly during restricted feeds, critical medium components have been stabilized, including FGF2 (also known as basic FGF; bFGF). As a result, TeSR™-AOF allows for both daily and restricted feeding schedules while maintaining cell quality and performance.

TeSR™-AOF is compatible with many culture matrices, including Corning® Matrigel® hESC-Qualified Matrix, Vitronectin XF™ (Catalog #07180), and CellAdhere™ Laminin-521 (Catalog #77003).

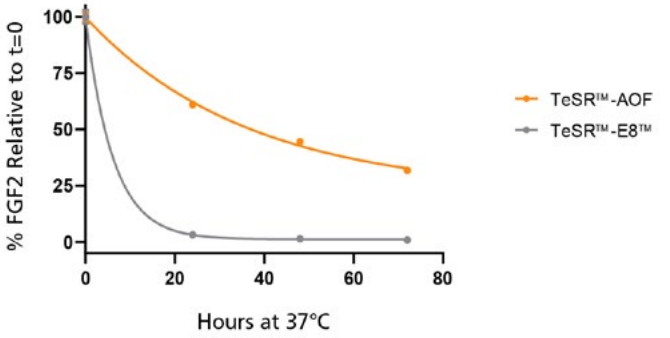
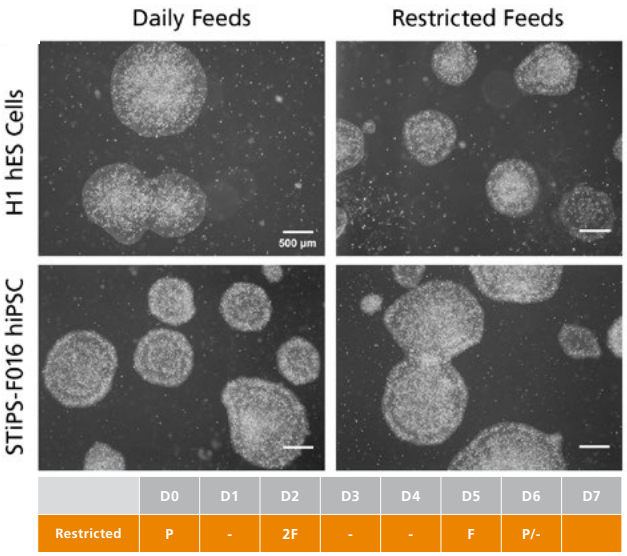


Figure 34. Native bFGF Levels Are Stabilized at 37°C in TeSR™-AOF

TeSR™-AOF and TeSR™-E8™ were incubated at 37°C for 24, 48, and 72 hours. FGF2 levels were measured by Meso Scale Discovery (MSD) immunoassay; data were normalized to t = 0 levels for TeSR™-E8™ and TeSR™-AOF, respectively. FGF2 levels in TeSR™-AOF remain at 36.7 ± 5.61% of t = 0 levels at 72 hours when incubated at 37°C. Data representative of n = 3 biological replicates ± SD.



	D0	D1	D2	D3	D4	D5	D6	D7
Restricted	P	-	2F	-	-	F	P/-	

Figure 35. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Exhibit Comparable Colony Morphology

hPSCs were maintained on Vitronectin XF™ for five passages. Phase-contrast images were taken on Day 7 after seeding. For restricted feeds, hPSCs were fed with a double volume (4 mL) of medium on Day 2 after passage, followed by two consecutive skipped days of feeds, with a final single-volume feed (2 mL) on Day 5, prior to passaging on Day 6 or 7. hPSCs maintained in TeSR™-AOF exhibit hPSC-like morphology, forming densely packed, round colonies with smooth edges.

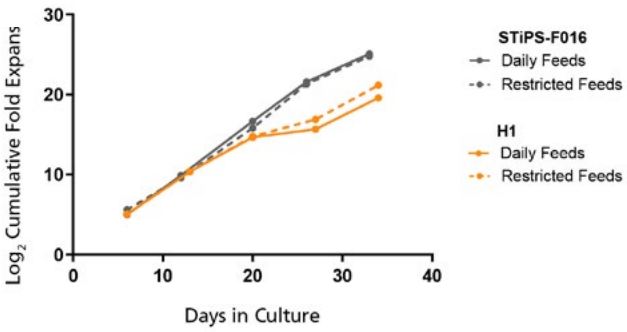


Figure 36. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Have Comparable Expansion Rates

hPSCs were maintained on Vitronectin XF™ for five passages. At the end of each passage, cell counts were obtained using the Nucleocounter® NC-200™ ChemoMetec automated cell counter to count DAPI-stained nuclei. The $\log_2$ transformed cumulative fold expansion was plotted against time in culture (days).

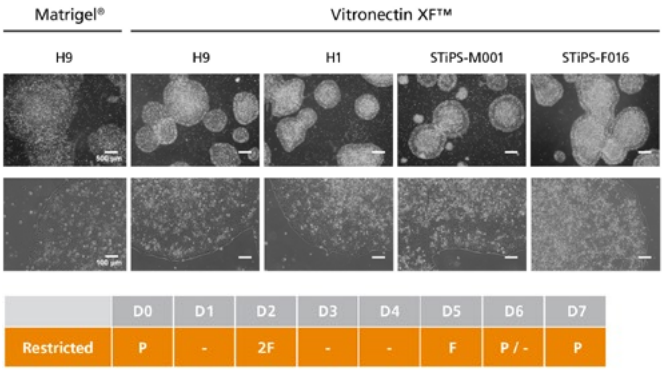


Figure 37. hPSCs Cultured in TeSR™-AOF with Restricted Feeding Demonstrate Classic hPSC Colony Morphology

hPSCs maintained in TeSR™-AOF were passaged as aggregates with ReLeSR™ passaging reagent every 6 - 7 days for more than 10 passages. hPSCs maintained in TeSR™-AOF exhibit hPSC-like morphology, forming densely packed, round colonies with smooth edge morphology. Homogeneous cell morphology characteristic of hPSCs are observed, including large nucleoli and scant cytoplasm.

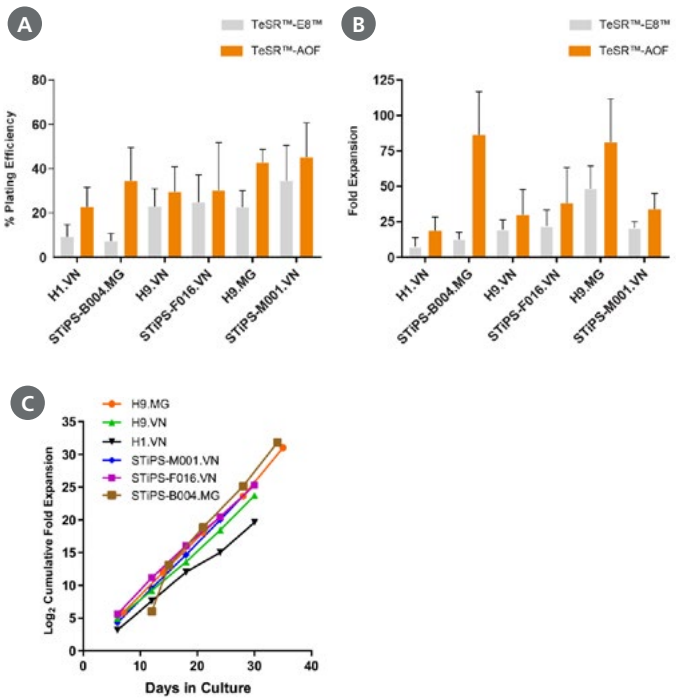


Figure 38. hPSCs Maintained in TeSR™-AOF Have Improved Attachment and Higher Overall Expansion Compared to Low-Protein Medium

(A) hPSCs cultured in TeSR™-AOF demonstrate a higher plating efficiency compared to hPSCs maintained in low-protein medium (TeSR™-E8™). Plating efficiency is calculated by seeding a known number of aggregates and comparing to the number of established colonies on Day 7. (B) hPSCs maintained in TeSR™-AOF exhibit a higher average fold expansion per passage compared to TeSR™-E8™. (C) hPSCs cultured in TeSR™-AOF demonstrate consistent expansion and minimal cell-line-to-cell-line variability between ES and iPS cell lines assessed. Cumulative fold expansion was measured from passage 1 to 5. Data represented as mean plating efficiency or fold expansion across 10 passages ± SD. MG = Matrigel®; VN = Vitronectin XF™.

Why Use TeSR™-AOF?

- Reduce risk in your choice of ancillary materials by selecting a medium with no animal raw materials to the secondary level of manufacturing
- Consistently culture hPSCs with high-quality colony morphology, robust attachment, and high cell expansion
- Enjoy the flexibility to use alternate feeding schedules without sacrificing cell quality, with stabilization of key components, including FGF2

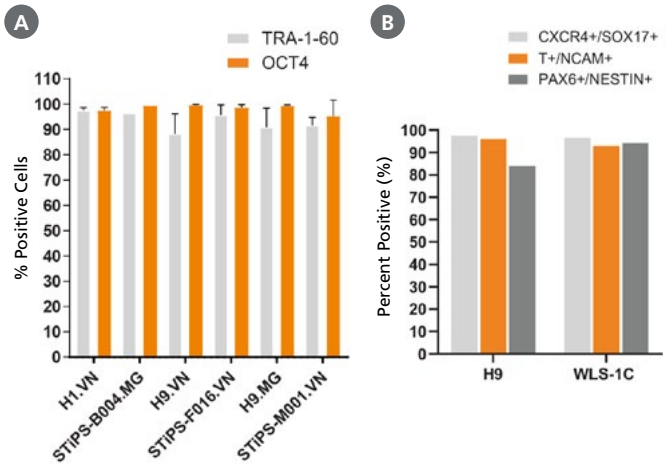


Figure 39. hPSCs Cultured in TeSR™-AOF Express Markers of the Undifferentiated State and Differentiate to the Three Germ Layers

(A) hPSCs maintained in TeSR™-AOF exhibit high levels of TRA-1-60 and OCT4 by flow cytometry at passage 5 and 10. Across n = 6 cell lines, the average TRA-1-60 expression was 93.4 ± 3.37%, and percentage of OCT4-positive cells was 98.3 ± 1.55%. Data shown represent cells at an average of passage 5, and 10 flow cytometry results for each cell line. MG = Matrigel®; VN = Vitronectin XF™.
(B) Efficient differentiation to the three germ layers was demonstrated in one hES and one hiPS cell line maintained for > 5 passages in TeSR™-AOF. Cultures were processed for flow cytometry and assessed for $CXCR4^+/SOX17^+$ cells on Day 5 following differentiation using the STEMdiff™ Definitive Endoderm Kit. Cultures were processed for flow cytometry and assessed for Brachyury $(T)^+/OCT4^-$ cells on Day 5 following differentiation in STEMdiff™ Mesoderm Induction Medium. Cultures were processed for flow cytometry and assessed for $PAX6^+/Nestin^+$ cells on Day 7 following monolayer differentiation using STEMdiff™ Neural Induction Medium.

Learn more at www.stemcell.com/TeSR-AOF

mTeSR™1

Maintain hPSCs in cGMP-Grade, Feeder-Free hPSC Medium

mTeSR™1 (Catalog #85850) is a serum-free and complete cell culture medium that has been used to successfully maintain thousands of hPSC lines, with established protocols for applications ranging from gene editing and bioreactor expansion to lineage-specific differentiation. mTeSR™1 is designed for use in cell therapy research applications, manufactured following the recommendations of USP <1043> on ancillary materials, and available for use under an approved US FDA Investigational New Drug (IND) application.

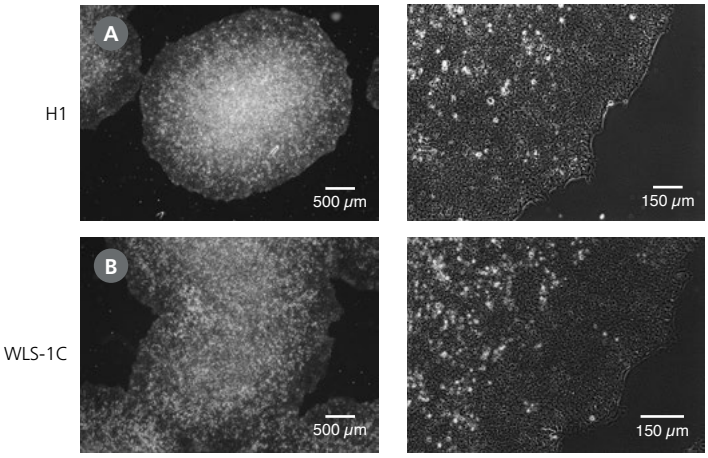


Figure 40. Normal Human ES and iPS Cell Morphology Is Observed in mTeSR™1 Cultures

Undifferentiated (A) H1 human ES and (B) WLS-1C human iPS cells cultured on Corning® Matrigel® matrix in mTeSR™1 retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type after 10 passages. Densely packed cells and multilayering are prominent when cells are ready to be passaged.

Learn more at www.stemcell.com/mTeSR1

TeSR™-E8™

Maintain hPSCs in Animal Component-Free, Feeder-Free hPSC Medium

TeSR™-E8™ (Catalog #05990) is based on the E8 formulation[4,11] developed by the laboratory of Dr. James Thomson, the lead research group behind the design of mTeSR™1. TeSR™-E8™ contains only the most critical components required for maintenance of hPSCs, providing a simpler medium for hPSC culture. This medium can be used with Vitronectin XF™ (Catalog #07180) for a completely xeno-free system.

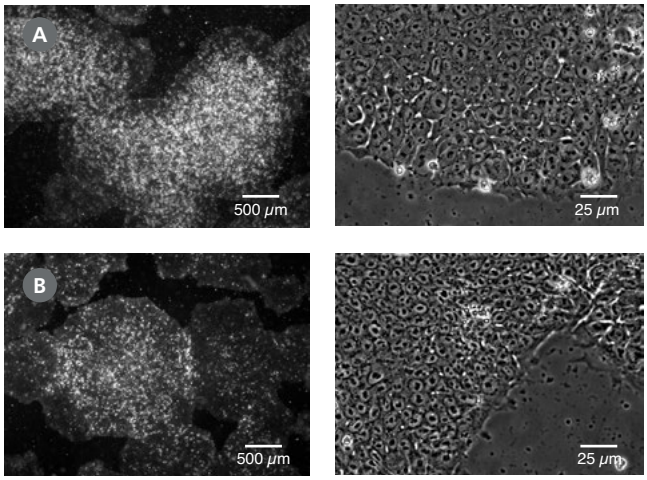


Figure 41. Normal Human ES and iPS Cell Morphology Is Observed in TeSR™-E8™ Cultures

Undifferentiated (A) human ES (H9) and (B) human iPS (WLS-1C) cells cultured on Corning® Matrigel® in TeSR™-E8™ retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type. Densely packed cells and multilayering are prominent when cells are ready to be passaged.

Learn more at www.stemcell.com/TeSR-E8

Fed-Batch Media and Scale-Up

Expand Large Numbers of hPSCs in 3D Suspension Culture

Suspension culture of hPSCs as 3D aggregates provides a convenient method to produce large numbers of high-quality, undifferentiated hPSCs with reduced labor and costs. hPSCs expanded in the TeSR™ family suspension culture systems have robust growth, maintain high expression of pluripotent stem cell markers, and retain trilineage differentiation ability.

Why Use Suspension Culture?

- Simplify your culture system with serum-free media that do not require microcarriers or external matrices
- Rapidly generate billions of hPSCs in as few as 2 - 3 weeks
- Save time and money with a fed-batch strategy that does not require full medium changes

Why Use TeSR™-AOF 3D?

- Minimize risk associated with your ancillary materials by selecting a medium with no animal raw materials to the secondary level of manufacturing
- Reduce time and labor with a fed-batch feeding strategy that does not require full-medium changes
- Scale up high-quality hPSCs rapidly without requiring adaptation from 2D culture

TeSR™-AOF 3D

Use TeSR™-AOF 3D (Catalog #100-0720) to safely generate large numbers of high-quality hPSCs for your cell banking and cell therapy manufacturing applications. TeSR™-AOF 3D has been designed to support rapid scale-up without requiring adaptation from 2D culture, while also reducing time and labor with a fed-batch feeding strategy that does not require full medium changes. And, as you might be thinking about the eventual therapeutic applications of your work, TeSR™-AOF 3D contains no materials of animal or human origin to at least the secondary level of manufacturing, eliminating the need for viral safety testing.

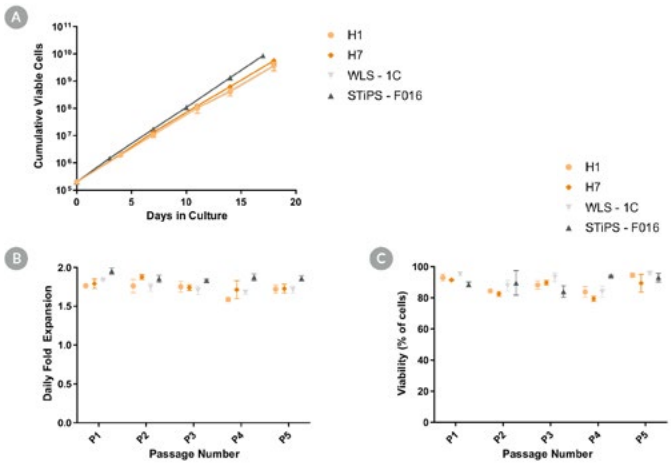


Figure 42. Growth of hPSCs in TeSR™-AOF 3D

TeSR™-AOF 3D supports hPSC expansion over multiple passages in aggregate suspension culture. Shown are (A) cumulative viable cells, (B) daily fold expansion, and (C) end-of-passage viabilities in human ESC (H1 and H7) and iPSC (WLS-1C and STiPS-F016) lines over 5 passages. Error bars represent ± SD, n = 3.

Learn more at www.stemcell.com/TeSR-AOF-3D

PBS-MINI Bioreactor

Rapidly Scale Up Your 3D hPSC Culture

Reliably and rapidly scale up your 3D cell cultures and suspensions with the PBS-MINI Bioreactor (Catalog #100-1005). The gentle yet efficient mixing provided by the Vertical-Wheel™ impeller enables the expansion of shear-sensitive cells without anti-foaming agents or shear protectants. Ideal for hPSCs cultured in the TeSR™ 3D family of media, the compact, sealed base unit and the 0.1 (Catalog #100-1006) and 0.5 (Catalog #100-1007) MAG Single-Use Vessels can be used inside incubators. Single-use vessels are also available with bottom ports (Catalog #100-1300 and #100-1301), facilitating easy access to the vessel's contents, and allowing cells and media to be drained directly from the bottom of the vessel. Conveniently control your culture system with a speed dial and digital display, and visualize cells in low-light conditions using built-in LED lights.

Learn more at www.stemcell.com/PBS-MINI

mTeSR™3D

Based on mTeSR™1, mTeSR™3D (Catalog #03950) is optimized for the expansion and scale-up of hPSCs. It is optimized as a fed-batch culture system, in which required nutrients are added daily, eliminating the need for daily medium exchanges.

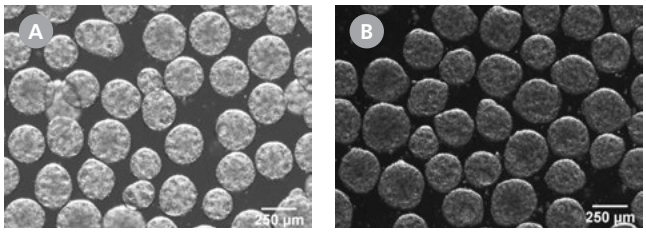


Figure 43. Morphology of hPSC Aggregates Cultured in mTeSR™3D

Characteristic morphology of suspension-cultured hPSC aggregates includes: approximately spherical shape, edges that are clear but not perfectly smooth, and a mottled or pock-marked appearance. Aggregates should be approximately 350 - 400 µm by the end of the passage. Shown are (A) human ES cell line H7 and (B) human iPS cell line STiPS-F016 cultured in mTeSR™3D.

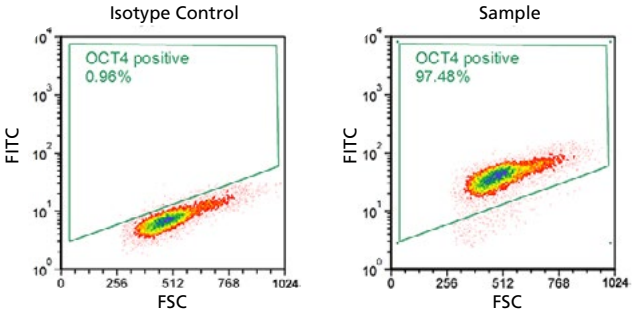


Figure 44. OCT4 Expression of hPSCs Cultured in mTeSR™3D

hPSCs expanded in mTeSR™3D maintain expression of pluripotent stem cell markers. Shown are representative plots of OCT4 expression after 7 passages in mTeSR™3D.

Learn more at www.stemcell.com/mTeSR3D

TeSR™-E8™3D

TeSR™-E8™3D (Catalog #3990) is a low protein, animal component-free medium based on TeSR™-E8™. The system contains only the most critical components for hPSCs, providing a simpler culture medium for robust, large-scale hPSC expansion. It uses a fed-batch feeding strategy that replenishes nutrients daily while reducing labor and costs.

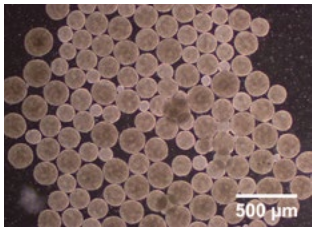


Figure 45. Morphology of hPSC Aggregates Cultured in TeSR™-E8™3D

Characteristic morphology of suspension-cultured hPSC aggregates includes: approximately spherical shape, edges that are clear but not perfectly smooth, and a mottled or pock-marked appearance. Aggregates should be approximately 350 - 400 µm by the end of the passage. Shown are human ES cell line H1 cultured in TeSR™-E8™3D.

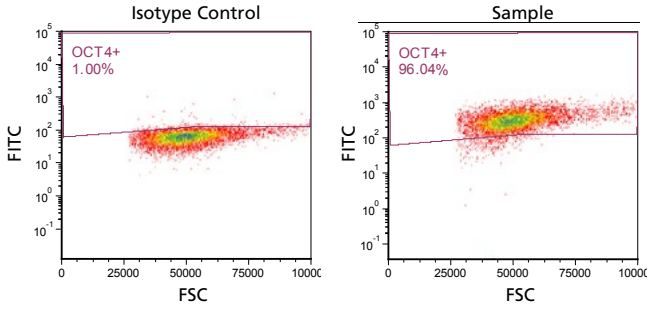


Figure 46. OCT4 Expression of hPSCs Cultured in TeSR™-E8™3D

hPSCs expanded in TeSR™-E8™3D maintain expression of pluripotent stem cell markers. Shown are representative plots of OCT4 expression after 10 passages in TeSR™-E8™3D.

Learn more at www.stemcell.com/TeSR-E8-3D

Naïve Induction and Maintenance

RSeT™ Feeder-Free Medium

Maintain Feeder-Free, Naïve-Like hPSCs in Defined Medium

RSeT™ Feeder-Free Medium (Catalog #05975) is a serum-free medium that reverts primed hPSCs and maintains cells in a naïve-like state without the need for basic fibroblast growth factor (bFGF) or feeder cells. RSeT™ Feeder-Free Medium produces robust cultures with a naïve-like morphology and increased expression of key naïve-associated transcripts. This improved formulation enables efficient reversion to a naïve-like state as early as passage 1, without the variability and burden associated with using feeder cells.

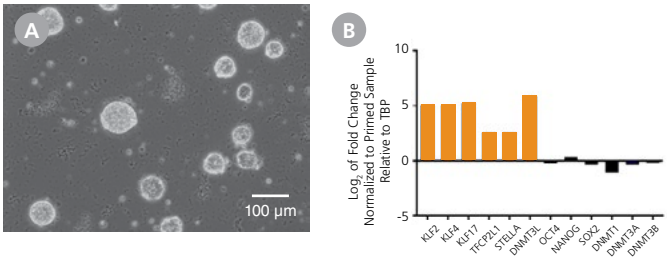


Figure 47. hPSCs Maintained in RSeT™ Feeder-Free Medium Are Reverted to a Naïve-Like State and Express High Levels of Naïve-Associated Genes

(A) A representative image of hPSCs that reverted to a naïve-like state after being cultured in RSeT™ Feeder-Free Medium for 1 passage. During reversion, colonies change from a flat morphology to a domed morphology characteristic of naïve-state hPSCs. (B) Expression of naïve-associated genes (KLF2, KLF4, KLF17, TFCP2L1, STELLA, and DNMT3L) in hPSCs that were reverted to a naïve-like state in RSeT™ Feeder-Free Medium. Expression levels were measured by qPCR and normalized to levels in primed hPSCs.

Why Use RSeT™ Feeder-Free Medium?

- Reproducibly maintain naïve-like hPSCs with a serum-free, bFGF-free formulation that contains pre-screened quality components
- Reduce cost and variability with easy-to-use, feeder-independent culture system
- Efficiently revert cells to a naïve-like state with stable domed morphology, naïve gene expression profiles, and low levels of spontaneous differentiation, without the need for exogenous genes

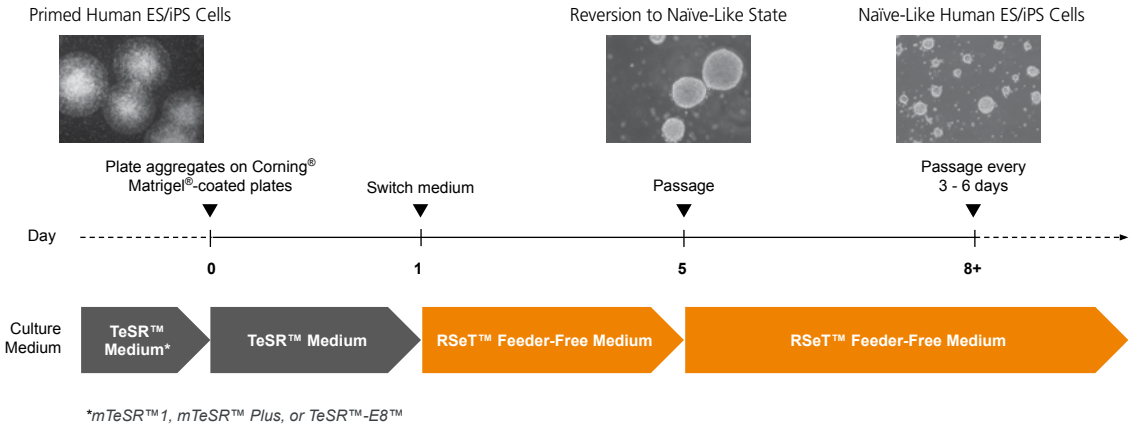


Figure 48. Schematic of Reversion of Primed to Naïve-Like hPSCs with RSeT™ Feeder-Free

Primed hPSCs are plated as aggregates in a TeSR™ medium (mTeSR™ Plus, mTeSR™1, or TeSR™-E8™). On Day 1, TeSR™ medium is replaced with RSeT™ Feeder-Free Medium, and the medium is exchanged every other day. By Day 4 or 5, the colonies are generally large enough to be passaged. During the initial culture in RSeT™ Feeder-Free Medium, colonies expand and begin to adopt a tightly packed, highly domed morphology characteristic of naïve-like stem cells with smooth and refractive colony edges as early as passage 1. Developed under license from the Weizman Institute of Science.[12]

Learn more at www.stemcell.com/RSeT-FeederFree

NaïveCult™

Achieve Serum- and Transgene-Free Induction and Expansion of Reset Naïve hPSCs

NaïveCult™ (Catalog #05580) is a serum-free media system that generates transgene-free, reset naïve hPSCs from primed hPSCs and allows for their continual maintenance. NaïveCult™ contains pre-screened quality components to work consistently across multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines.

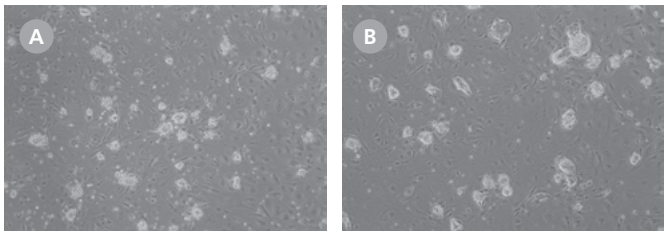


Figure 49. Human ES and iPS Cells Cultured in NaïveCult™ Show Characteristic Morphology of Naïve-State hPSCs

Representative images of human (A) H9 ES cells at passage 7 and (B) WLS-1C iPS cells at passage 9 that were reverted to a naïve state using the NaïveCult™ Induction Kit and subsequently cultured in NaïveCult™ Expansion Medium. During reversion, colonies change from a flat morphology to a tightly packed and uniformly domed morphology with refractive edges characteristic of naïve-state hPSCs.[13-15]

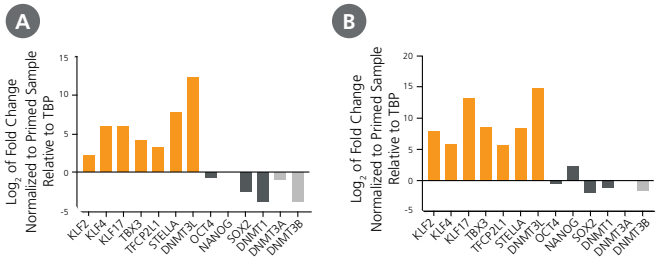
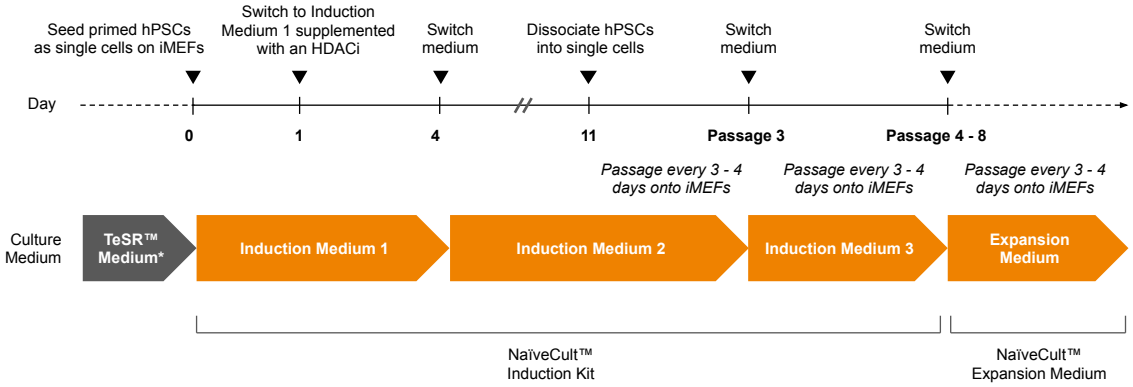


Figure 50. hPSCs Cultured in the NaïveCult™ Media System Express High Levels of Factors Associated with Naïve hPSCs[12-14]

Human (A) H9 ES cells and (B) WLS-1C iPS cells were reverted using the NaïveCult™ Induction Kit and maintained in NaïveCult™ Expansion Medium. Expression levels were measured by quantitative PCR (qPCR) and normalized to levels in primed hPSCs.



Note: From Day 0 onward, culture under hypoxic conditions (5% O_2, 5% CO_2). Perform full medium changes daily.
**mTeSR™1, mTeSR™ Plus, or TeSR™-E8™*

Figure 51. Schematic of Reversion of Primed to Naïve-Like hPSCs in NaïveCult™

Primed hPSCs are plated on irradiated mouse embryonic fibroblasts (iMEFs) and treated with ROCK inhibitor (10 µM Y-27632) for 24 hours in hypoxic conditions. Background differentiation will decrease between passage 3 and 8. At this time, cells can be transferred into NaïveCult™ Expansion Medium for long-term maintenance and expansion. Developed under license from Cambridge Enterprises.[13]

Learn more at www.stemcell.com/NaiveCult

hPSC Naïve State qPCR Array

The hPSC Naïve State qPCR Array (Catalog #07521) provides a validated 90-gene assay to characterize the state of hPSCs in the spectrum from naïve to primed pluripotency. Data analysis is streamlined with our flexible online app (**www.stemcell.com/qPCRanalysis**).

Learn more at www.stemcell.com/naive-array

Matrices

Cell culture matrices support the growth and differentiation of hPSCs by mimicking the in vivo extracellular matrix. When used with TeSR™ maintenance media, Vitronectin XF™ and CellAdhere™ Laminin-521 provide a robust culture system for feeder-free cell maintenance.

Vitronectin XF™

Serum- and Feeder-Free Maintenance and Differentiation of hPSCs

Vitronectin XF™ (Catalog #07180), developed and manufactured by Nucleus Biologics, is a defined, xeno-free cell culture matrix that supports the growth and differentiation of hPSCs. Use with mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), TeSR™-E8™ (Catalog #05990), or TeSR™-AOF (Catalog #100-0401) medium for a completely defined culture system that allows for complete control over the culture environment and provides more consistent, reproducible results in downstream applications. Human ES and iPS cells cultured on Vitronectin XF™ retain pluripotency and normal colony morphology, without the need for an adaptation step. Pair with Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0485) or ReLeSR™ (Catalog #05872) when passaging to maintain high-quality cultures.

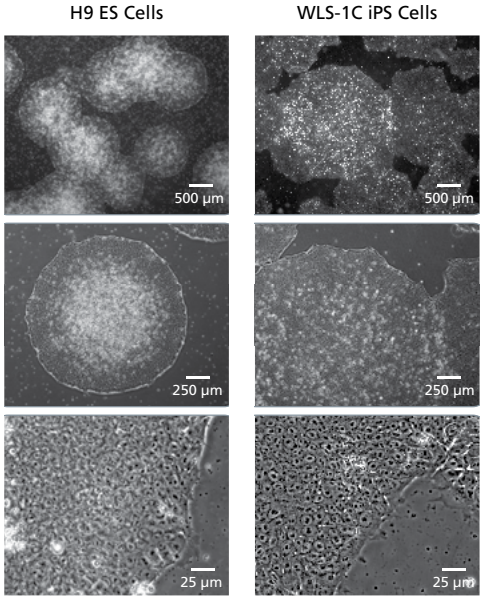


Figure 52. Human ES and iPS Cells Exhibit Normal Morphology When Cultured on Vitronectin XF™ Cell Culture Matrix in TeSR™-E8™

Undifferentiated human ES (H9) and iPS (WLS-1C) cell cultures exhibit normal morphology when cultured on Vitronectin XF™. Colonies are round, tightly packed, and multilayered, with a high nucleus-to-cytoplasm ratio. Cells were transferred directly from Matrigel® hESC-Qualified Matrix without an adaptation step.

Note: Colonies grown in TeSR™-E8™ have a more condensed and round morphology when grown on Vitronectin XF™ matrix, compared to colonies grown on Matrigel® hESC-Qualified Matrix.

Learn more at www.stemcell.com/Vitronectin-XF

Why Use Vitronectin XF™?

- Decrease sources of variability in your experiment with a recombinant human protein matrix
- Handle at room temperature without matrix gelling
- Use with any TeSR™ family medium to maintain hPSCs
- Create a completely xeno-free system when used with TeSR™-E8™ or TeSR™-AOF

CellAdhere™ Laminin-521

Long-Term Feeder-Free Maintenance of hPSCs

CellAdhere™ Laminin-521 (Catalog #77003) is a defined and xeno-free cell culture matrix that supports the feeder-free growth and differentiation of hPSCs. Laminin 521 is naturally expressed and secreted by hPSCs in the inner cell mass of the embryo, therefore creating a biologically relevant hPSC culture environment in vitro.

For consistent, reproducible results in downstream applications, use CellAdhere™ Laminin-521 with TeSR™ maintenance media. Compared to other matrices, CellAdhere™ Laminin-521 increases single-cell attachment and survival without requiring apoptotic inhibitors during plating. For single-cell passaging, use CellAdhere™ Laminin-521 with eTeSR™ (Catalog #100-1215) maintenance medium.

Note: If passaging hPSCs as single cells, check the karyotype frequently for genetic aberrations.

Why Use CellAdhere™ Laminin-521?

- Decrease sources of variability in your experiment with a recombinant human protein matrix
- Passage cells without the need for apoptotic inhibitors
- Use with any TeSR™ family medium to maintain hPSCs
- Increase single-cell attachment and survival when using with eTeSR™ for single-cell passaging
- Mimic the stem cell niche with this physiologically-relevant matrix

Learn more at www.stemcell.com/Laminin-521

Dissociation Reagents

ReLeSR™ and Gentle Cell Dissociation Reagent

Passage hPSCs, Enzyme-Free

ReLeSR™ (Catalog #05872) selectively detaches undifferentiated cells from hPSC cultures, eliminating manual selection and scraping. Passaging hPSCs with ReLeSR™ enables the easy generation of optimally sized aggregates while eliminating the hassle and variability of manual selection. By removing the need for scraping, ReLeSR™ more readily enables the use of culture flasks and other closed vessels, thus facilitating culture scale-up and automation

Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0485) is an enzyme-free reagent suitable for the dissociation of hPSCs into cell aggregates for routine passaging or into a single-cell suspension.

ReLeSR™ and GDCR are both manufactured under relevant cGMPs and can be used as part of a high-compliance hPSC maintenance workflow, such as with TeSR™-AOF (Catalog #100-0401) medium and CellAdhere™ Laminin-521 (Catalog #77003).

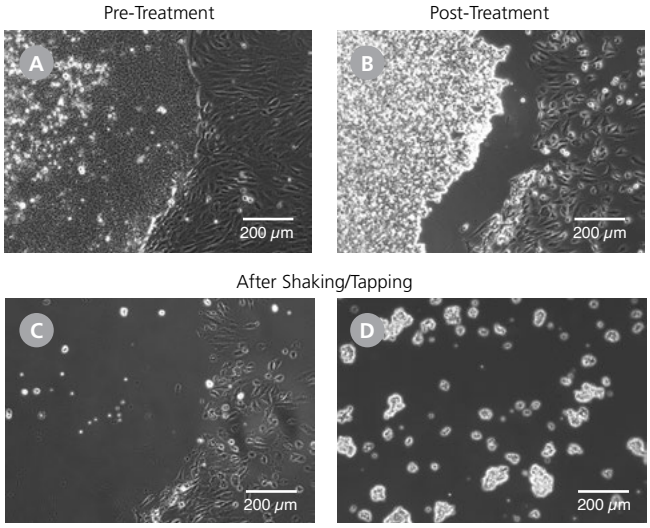


Figure 53. ReLeSR™ Selectively Detaches Undifferentiated Cells from hPSC Cultures Without Manual Selection and Generates Optimally Sized Aggregates

(A) An hPSC culture ready for passaging. Note the presence of differentiated cells at the edge of the undifferentiated hPSC colony. (B) Following incubation with ReLeSR™, the undifferentiated hPSC colony starts to lift off of the cultureware. The differentiated cells remain attached to the cultureware. (C) Following shaking/tapping of the cultureware, the undifferentiated cells completely lift off of the cultureware. (D) The undifferentiated hPSC colony is broken up into optimally sized aggregates for replating.

Learn more at www.stemcell.com/ReLeSR

Learn more at www.stemcell.com/GCDR

ACCUTASE™

For Creation of Single-Cell Suspensions

Use ACCUTASE™ (Catalog #07920) for routine cell detachment from standard tissue culture plasticware and adhesion-coated plasticware. This solution of proteolytic and collagenolytic enzymes comes ready-to-use and has been shown to work effectively across a wide variety of cell types. Formulated at a lower concentration than conventional trypsin, this gentle alternative preserves cell surface epitopes for downstream flow cytometry analyses and ensures high cell viability, without the need for a neutralizing solution. ACCUTASE™ does not contain mammalian or bacterial-derived products.

Why use ACCUTASE™?

- Obtain cleaner cultures with a ready-to-use solution, free from mammalian and bacterial-derived products
- Achieve efficient cell detachment with this gentle trypsin alternative
- Ensure high cell viability, without the need for a neutralizing solution

Protocol
Enzyme-Free Passaging of hPSCs Using ReLeSR™

Protocol
Enzyme-Free Passaging of hPSCs Using Gentle Cell Dissociation Reagent

Gene Editing

CloneR™2

Enhance the Cloning Efficiency and Single-Cell Survival of hPSCs

Generate clonal hPSC lines that maintain their genomic integrity and downstream differentiation potential with this defined, serum-free supplement. CloneR™2 (Catalog #100-0691) increases the cloning efficiency and survival of human ESCs and iPSCs under high-stress conditions, including seeding at low or high densities, post-thaw recovery, and when creating monolayers ahead of downstream differentiation. When gene editing, add CloneR™2 to improve hPSC survival after electroporation and during clonal deposition.

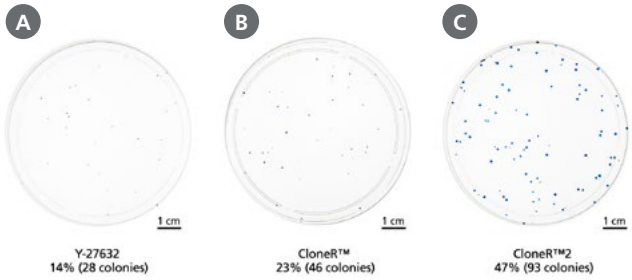


Figure 54. CloneR™2 Improves Cloning Efficiency and Colony Size

hPSCs display a considerable increase in cloning efficiency when cloned using (B) CloneR™ compared to using (A) Y-27632 compound. (C) CloneR™2 further improves cloning efficiency and increases colony size when compared to either Y-27632 compound or CloneR™. Shown are examples of H9 hESCs in 10-cm dishes, plated at 200 cells per dish (~4 cells/cm²) in mTeSR™ Plus on Vitronectin XF™.

Why Use CloneR™2?

- Generate more colonies for selection days sooner
- Consistently generate clones with similar high performance across culture systems and cell lines
- Plate your cells more efficiently at all densities and after high-stress events such as electroporation or thawing
- Save time by going straight to single cells, with no adaptation phase required

Learn more at www.stemcell.com/CloneR2

Webinar

Facilitating hPSC Single Cell Seeding Workflows Using CloneR™2

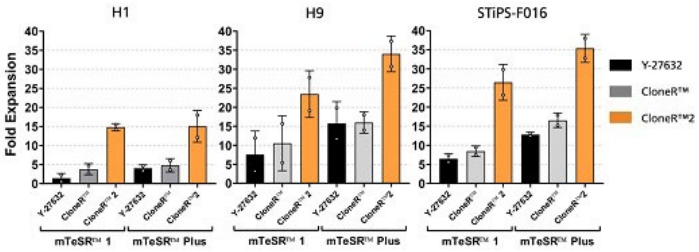


Figure 55. CloneR™2 Improves Recovery After Electroporation

Three hPSC lines were electroporated, then plated in mTeSR™1 and mTeSR™ Plus containing Y-27632, CloneR™, or CloneR™2. After 24 hours, cells were maintained in TeSR™ media (without cloning supplement) and analyzed on day 5. In all 3 cell lines, CloneR™2 dramatically improved cell survival and expansion compared to Y-27632 and CloneR™ (n = 2 replicates per cell line).

Also Consider: CloneR™

CloneR™ (Catalog #05888) is the original serum-free supplement formulated for enhancing the cloning efficiency and single-cell survival of hPSCs, especially under clonal and low-density seeding conditions.

ArciTect™

Genome Edit hPSCs with a CRISPR-Cas9 System

The ArciTect™ product family is a ribonucleoprotein (RNP)-based CRISPR-Cas9 genome editing system for hPSCs. Whether you are seeking purified Cas9 proteins, customizing guide RNA targeting, estimating editing efficiency, or optimizing transfection protocols, the ArciTect™ toolkit contains qualified solutions for every step in the hPSC genome editing workflow. Refer to the Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084) for a protocol optimized and validated for use with ArciTect™.

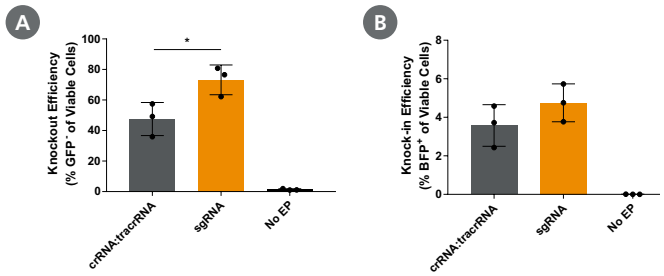


Figure 56. Efficient Genetic Knockout and Knock-In in Human Pluripotent Stem Cells Using the ArciTect™ CRISPR-Cas9 System

1C-eGFP hPSC lines were cultured in mTeSR™1 supplemented with CloneR™ for 24 hours after electroporation with CRISPR-Cas9 RNP complexes containing ArciTect™ Cas9 Nuclease and either ArciTect™ crRNA:tracrRNA duplexes or sgRNA targeting GFP, co-delivered with ssODN encoding nucleotides to convert GFP to BFP. (A) Knockout (% GFP- cells) and (B) knock-in (% BFP+ cells) efficiency were measured by flow cytometry 3 days after electroporation; n = 3. Control samples were not electroporated (no EP). Error bars represent standard deviation.

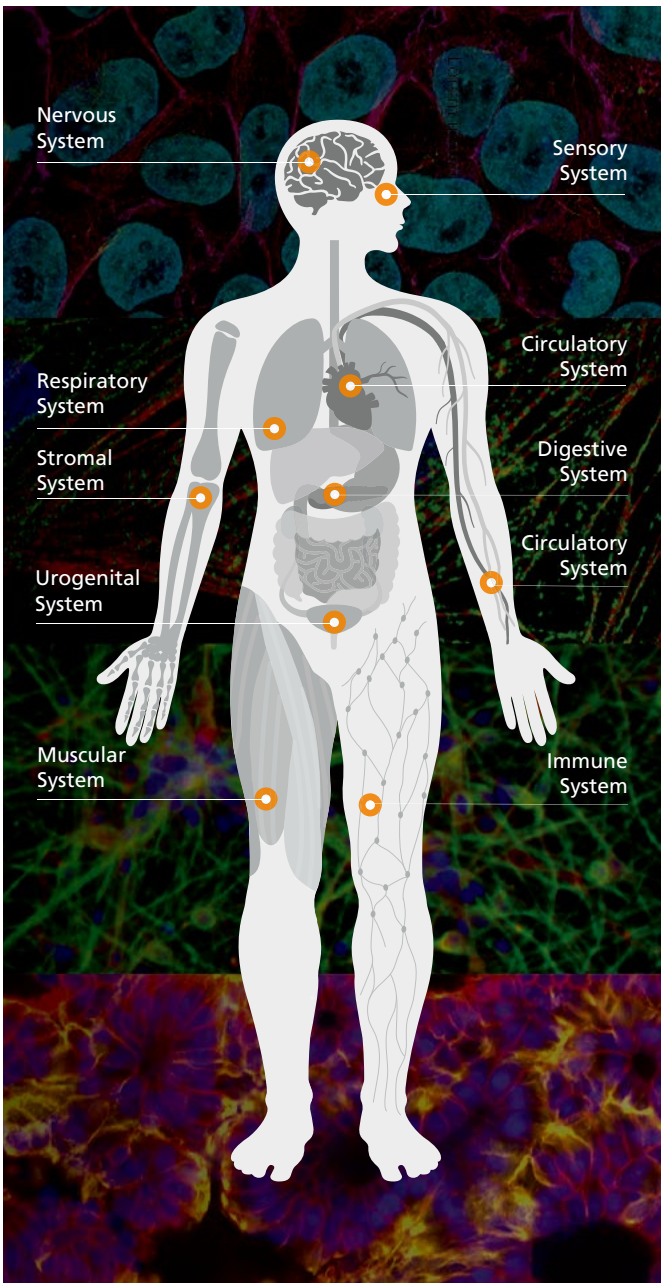
Learn more at www.ArciTect.com

Differentiation

STEMdiff™ Pluripotent Stem Cell Differentiation Media

Consistent human pluripotent stem cell (hPSC) differentiation is pivotal to high-quality results. Without standardized hPSC culture conditions, even the most detailed and rigorously followed stem cell differentiation protocols may still lead to inconsistent differentiation.[16-17] Use STEMdiff™—a line of culture medium kits specifically optimized for hPSC differentiation—to reproducibly differentiate multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines to 2D cell types and 3D organoid models originating from all three embryonic germ layers.

Each easy-to-use kit comes with detailed, user-friendly protocols to standardize your differentiation protocols. For gene-edited or patient-derived hPSC lines, these optimized media and protocols enable the generation of a variety of cell types with the same genotype. The STEMdiff™ family of products is part of our complete system of reagents for hPSC culture and is compatible with TeSR™ maintenance media.



Why Use STEMdiff™?

- Reduce experimental variability with formulations optimized under rigorous quality controls
- Differentiate across multiple ES and iPS cell lines
- Standardize your differentiation to cells from all three germ layers with simplified kit formats
- Generate and bank progenitor cell types for experimental flexibility or as a reliable cell source for customized downstream differentiation

Tools for hPSC Differentiation

Explore the 40+ cell types and organoids you can generate with STEMdiff™ hPSC differentiation kits in our hPSC Differentiation Brochure.

Learn more at www.STEMdiff.com

Customizable Differentiation

STEMdiff™ APEL™2

STEMdiff™ APEL™2 Medium (Catalog #05270) is a fully defined, serum-free, and animal origin-free (AOF) medium for the differentiation of hPSCs. It is based on the APEL formulation published by Ng et al.[18] and lacks undefined components such as protein-free hybridoma medium. This medium can be used in adherent or embryoid body (EB)-based protocols, such as with AggreWell™ plates (see page 33). Appropriate induction factors must be added before use.

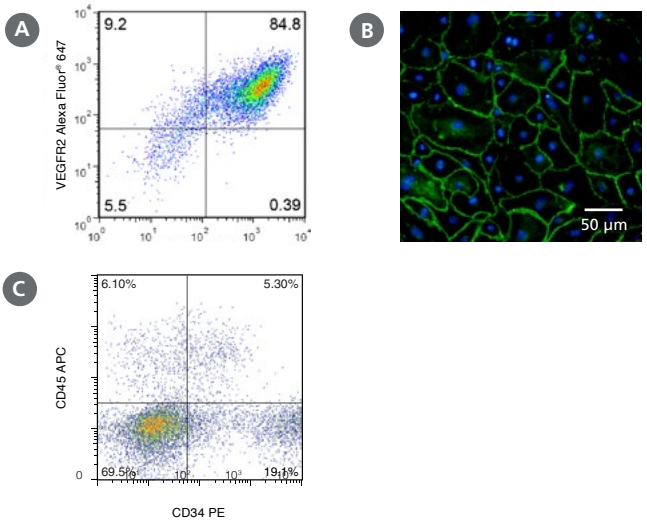


Figure 57. STEMdiff™ APEL™ Media Can Be Used for Customized Differentiation to Various Mesodermal Cell Lineages

(A) Endothelial differentiation of STiPS-F001 iPSCs using STEMdiff™ APEL™ medium*, based on methods by Tan et al.[19] (B) Immunocytochemistry image of CD31 (green; nuclei in blue) in endothelial cells differentiated from H1 ESCs using STEMdiff™ APEL™. Image courtesy of the Cao Tong lab, University of Singapore. (C) Hematopoietic differentiation of H9 ESCs, based on methods by Ng et al.[18] and Chadwick et al.[20] with these changes: (1) STEMdiff™ APEL™ was used as the basal medium; (2) prior to differentiation, cells were maintained in mTeSR™1 on Matrigel®; (3) differentiation was performed on a Matrigel®-coated surface versus an EB-based method.

*STEMdiff™ APEL™ has been updated to STEMdiff™ APEL™2, which lacks undefined components such as protein-free hybridoma medium.

Why Use STEMdiff™ APEL™2?

- Ensure defined growth with this AOF formulation
- Tailor your differentiation protocols to your specific cells using this robust and published basal medium
- Differentiate to a variety of cell lineages, including hematopoietic, endothelial, and epithelial
- Benefit from versatility with adherent- or EB-based protocols

TeSR™-E5 and TeSR™-E6 Media

TeSR™-E5 (Catalog #05916) and TeSR™-E6 (Catalog #05946) are defined, serum-, and xeno-free media that are based on the formulation of TeSR™-E8™, but do not contain transforming growth factor b (TGFb) or basic fibroblast growth factor (bFGF). Additionally, TeSR™-E5 does not contain insulin. These formulations may be used as basal media for differentiation of human ES and iPS cells, or other applications where removal of the above cytokines and insulin is desirable.

Cytokines and Recombinant Proteins

Need cytokines for your differentiation protocol?

Achieve high-quality cultures and reproducible results with premium reagents fit for your research.

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Learn more at www.stemcell.com/APEL2

Specialty Cultureware

AggreWell™ Plates

Reproducible Production of Uniform Embryoid Bodies

Many hPSC differentiation protocols begin with the formation of embryoid bodies (EBs). Conventional EB formation methods[21] create EBs that are heterogeneous in size and shape (Figure 58A), leading to inefficient and uncontrolled differentiation.[22]

AggreWell™ plates provide an easy and standardized approach to EB formation. Each well contains microwells of defined size, making it easy to produce large numbers of highly uniform EBs (Figure 54B) and to ensure reproducible differentiation.[23]

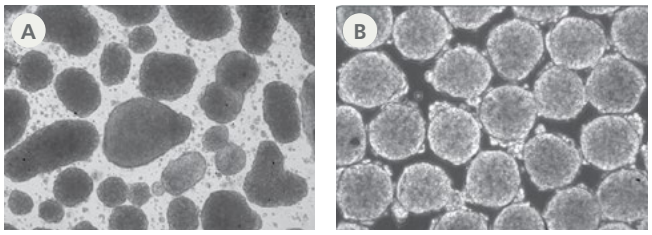


Figure 58. AggreWell™ Plates Are Used to Generate Uniform EBs

(A) EBs formed using conventional methods are heterogeneous in size and shape. (B) EBs formed using AggreWell™ plates are uniform in size and spherical in shape. Shown are EBs generated with 2000 cells using AggreWell™400.

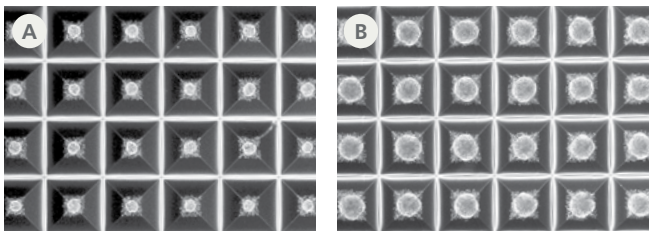


Figure 59. The Size of EBs Can Be Controlled in AggreWell™

Starting from single-cell suspension, hPSCs form EBs after 24 hours in AggreWell™. EB size is easily modified by adjusting the seeding density. Shown are EBs seeded at (A) 250 and (B) 1000 cells/microwell in AgreWell™400.

Learn more at www.stemcell.com/AggreWell

CellSTACK®

Large-Scale Expansion in 2D Monolayer Culture

Providing a useful alternative to multiple roller bottles or spinner flasks, CellSTACK® by Corning® (Catalog #38075) is ideal for cell culture scale-up, producing an average yield of 6.36 x 10^7 cells per chamber. Each chamber has 636 cm^2 of cell growth area and each stack has two 26 mm diameter filling ports with direct access to the bottom of the chamber, providing greater flexibility for sterile filling and emptying. The filling ports have standard 33 mm threaded caps, with non-wettable 0.2 µm pore membranes that are sealed directly to the caps to allow gas exchange while minimizing the risk of contamination. Made of sterilized, non-pyrogenic polystyrene with high optical clarity, CellSTACK® comes in 1-, 2-, 5-, or 10-chamber formats.

Learn more at www.stemcell.com/cellstack

Cultureware and General Supplies

Looking for more cultureware and lab supplies? We've got you covered. Visit our website to view our complete product listing.

Product Listing
www.stemcell.com/cultureware

AggreWell™ is available in 2 sizes of microwells: 400 µm (AggreWell™400) or 800 µm (AggreWell™800).

Product	Microwell Size	Cell Range	Plate format	Number of Embryoid Bodies	Catalog #
AggreWell™400	400 µm	50 - 3000 cells per EB	24-well plate	~ 1200 per well	34411/34415
			6-well plate	~ 5900 per well	34421/34425
AggreWell™800	800 µm	3000 - 20,000 cells per EB	24-well plate	~ 300 per well	34811/34815
			6-well plate	~1500 per well	34821/34825

Anti-Adherence Rinsing Solution (Catalog #07010) is required for optimal performance.

Courses and Training Support

Learn Firsthand from Our Experienced Scientists

Performing an unfamiliar laboratory technique can be challenging. Protocols are often lengthy and complicated, and inexperience may contribute to user or experimental errors. Increase your chances of success and perform your experiments with confidence by enrolling in one of our training programs before you begin.

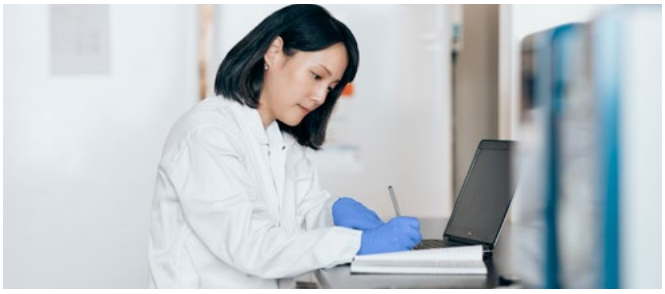
On-Demand Training

Access our free, self-paced, online training courses

Learn the techniques and protocols to move your research forward. Recorded lectures, step-by-step instructional videos, and libraries of curated resources will help guide you through entire workflows. Topics include hPSC maintenance and cell quality, neural induction, and expansion of hPSCs in 3D suspension culture. From passaging to cryopreservation, learn fundamental laboratory skills from the comfort of your own home.

Selected On-Demand Courses

- hPSC Quality & Maintenance
- Expansion of hPSCs in 3D Suspension Culture
- Neural Induction
- Human Intestinal Organoids
- Human Hepatic Organoids
- Pulmonary Culture



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Join from anywhere in the world to watch real-time video demonstrations, participate in online workshops, and learn from our team of scientific experts in our interactive, virtual training courses. Learn techniques and protocols to successfully derive and maintain high-quality human iPS cells from somatic cells and differentiate them toward specialized cell types in a virtual and interactive setting.

Selected Live-Virtual Courses

- hPSC Quality & Maintenance
- Reprogramming Somatic Cells to iPSCs
- Genome Editing hPSCs
- Human Pulmonary Organoid Culture
- Human Intestinal Organoids
- Customized Training

STEMCELL also offers in-person training to support the culture of hPSCs and their differentiation toward cerebral organoids, intestinal organoids, cardiomyocytes, or hematopoietic progenitors.

Learn more at www.stemcell.com/psc-training

Methods Library

Browse a curated library of detailed protocols, tips and tricks, video demonstrations, and more—to help you move your research forward faster.

Product and Scientific Support

Our dedicated team of experts is always ready to support you, whether you're troubleshooting a problem or looking for opportunities to collaborate.

References

1. Ludwig TE et al. (2023) Stem Cell Rep 18(9): 1744–52.
2. International Stem Cell Banking Initiative (2009) Stem Cell Rev Rep 5(4): 301–14
3. Schlaeger TM et al. (2015) Nat Biotechnol 33(1): 58–63.
4. Chen G et al. (2011) Nat Methods 8(5): 424–9.
5. Fujioka T et al. (2004) Int J Dev Biol 48(10): 1149–54.
6. Ha SY et al. (2005) Hum Reprod 20(7): 1779–85.
7. Ji L et al. (2004) Biotechnol Bioeng 88(3): 299–312.
8. Ware CB et al. (2005) Biotechniques 38(6): 879–80, 882–3.
9. Ludwig TE et al. (2006) Nat Methods 3(8): 637–46.
10. Ludwig TE et al. (2006) Nat Biotechnol 24(2): 185–7.
11. Beers J et al. (2012) Nat Protoc 7(11): 2029–40.
12. Gafni O et al. (2013) Nature 504(7479): 282–6.
13. Guo G et al. (2017) Development 144(15): 2748–63.
14. Takashima Y et al. (2014) Cell 158(6): 1254–69.
15. Guo G et al. (2016) Stem Cell Reports 6(4): 437–46.
16. D'Amour KA et al. (2005) Nat Biotechnol 23(12): 1534–41.
17. Katman SJ et al. (2011) Cell Stem Cell 8(2): 228–40.
18. Ng ES et al. (2008) Nat Protoc 3(5): 768–76.
19. Tan JY et al. (2013) Stem Cells Dev 22(13): 1893–906.
20. Chadwick K et al. (2003) Blood 102(3): 906–15.
21. Kurosawa H (2007) J Biosci Bioeng 103(5): 389–98.
22. Bauwens CL et al. (2008) Stem Cells 26(9): 2300–10.
23. Ungrin MD et al. (2008) PLoS One 3(2): e1565.

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