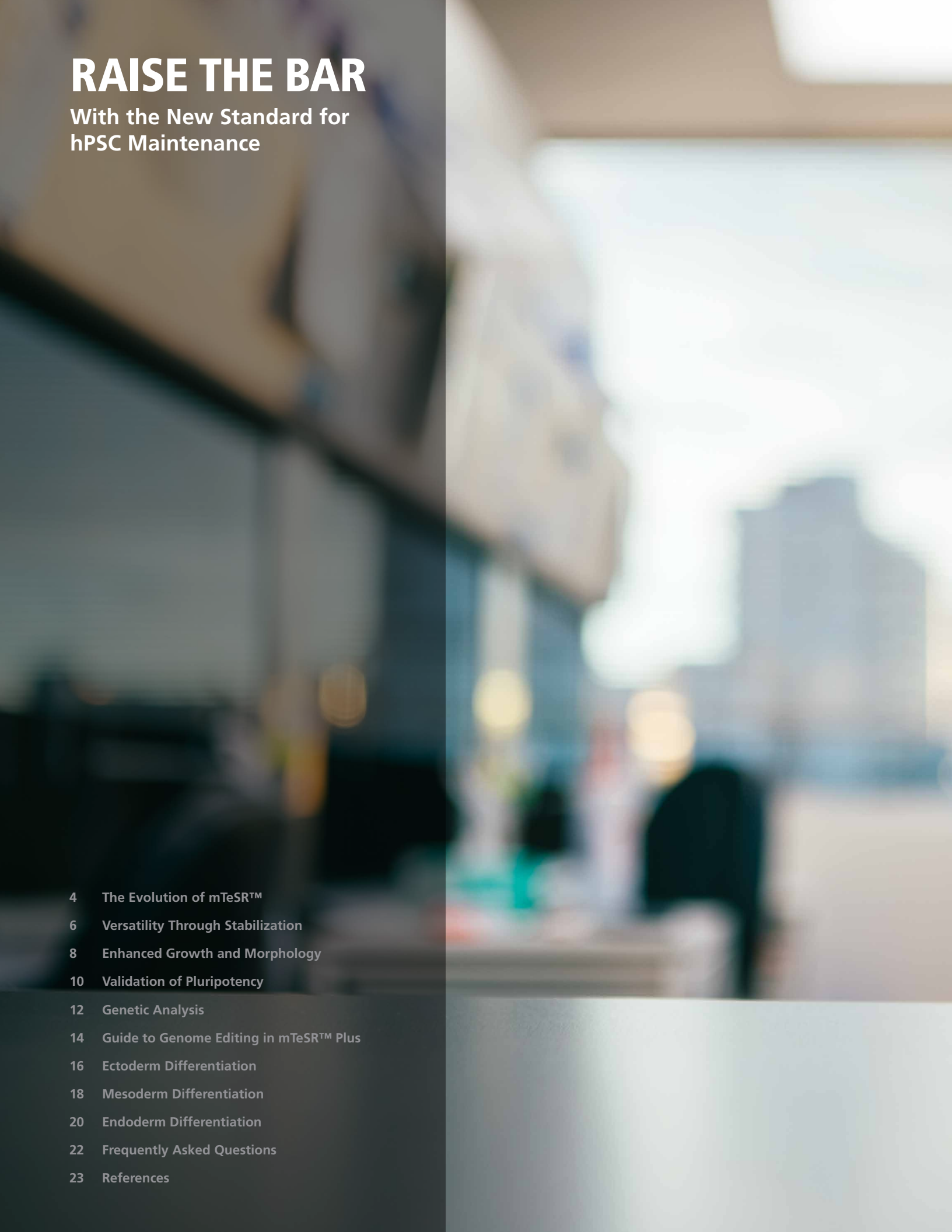
mTeSR™ Plus

THE NEW STANDARD

STEMCELL™
TECHNOLOGIES

RAISE THE BAR

With the New Standard for hPSC Maintenance



Why Use mTeSR™ Plus?

STABILIZED.
Enhanced buffering and stabilized components, including FGF2, support cell quality while allowing for alternate feeding schedules.

ENHANCED GROWTH.
Supports superior culture morphology and cell growth characteristics.

EFFICIENT CLONING.
Enables heightened single-cell survival when supplemented with CloneR™.

STREAMLINED.
Fully compatible with established genome editing and differentiation protocols.

REGULATED.
Manufactured under relevant cGMPs with enhanced documentation, including a viral safe profile to support cell therapy research.

THE EVOLUTION OF mTeSR™

"TeSR1" was created in the laboratory of Prof. James Thomson at the University of Wisconsin-Madison in 2006 by Dr. Tenneille Ludwig and colleagues. This was the first feeder-independent cell culture medium for human embryonic stem (ES) cells containing protein components derived solely from recombinant sources or purified from human material. First manufactured for commercial sale by STEMCELL Technologies in 2007, "modified" TeSR1 (mTeSR™1) went on to become the global standard for feeder-free human pluripotent stem cell (hPSC) maintenance, supporting thousands of scientific publications and discoveries in the stem cell research field.

1998

The first human ES cell lines are derived in Prof. James Thomson's laboratory at the University of Wisconsin-Madison. Cells are maintained in undefined serum-containing medium on a bed of mouse embryonic fibroblast (MEF) "feeder" cells.[1]

2000

Michal Amit and colleagues discover that, in the absence of serum, FGF2 is required to maintain human ES cells in the undifferentiated state.[2]

2001

Chunhui Xu establishes the first feeder-free method for human ES cell maintenance using MEF-conditioned medium.[3]

2006

Researchers in the James Thomson laboratory report the first feeder-independent human ES cell culture medium, "TeSR1", containing protein components derived solely from recombinant sources or purified from human material.[4]

2007

Shinya Yamanaka demonstrates the generation of induced pluripotent stem (iPS) cells from adult human dermal fibroblasts with four factors: Oct3/4, Sox2, Klf4, and c-Myc.[5]

2011

Researchers in the James Thomson laboratory develop E8™ medium, a minimal formulation containing only the critical components required for hPSC maintenance.[6] STEMCELL Technologies went on to release "TeSR™-E8™" in 2013.

2013

Feng Zhang describes Cas9-mediated genome editing in human ES cells maintained in mTeSR™1.[7]

2019

mTeSR™ Plus is developed with enhanced stability to ensure cell quality while allowing for truly versatile hPSC maintenance.

2020

mTeSR™ Plus is manufactured under relevant cGMPs using the same high-quality critical raw materials as the original, non-cGMP product. With the added benefits of enhanced critical raw material traceability, processes, and quality control validations, mTeSR™ Plus now enables a seamless transition from basic research to drug and cell therapy development.

Research & Development, Pluripotent Stem Cell Biology

As scientists, we know that there's nothing more important than having results that speak the truth. Supporting the work of hPSC researchers over the past decade has inspired us to continue innovating the highest quality of tools available — so that you always have confidence while pursuing your next breakthrough.

VERSATILITY THROUGH STABILIZATION

Medium color indicates the pH difference after hPSCs were cultured for 72 hours without feeding in either mTeSR™1 (left) or mTeSR™ Plus (right). For each medium, cells were at a density of approximately 2 x 10^6 cells per well of a 6-well plate at the end of the culture period.

Work Smarter, Not Harder.
Free up your days with just two golden rules, and nothing more.

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

Sustained pH and Stabilized Components, Including FGF2

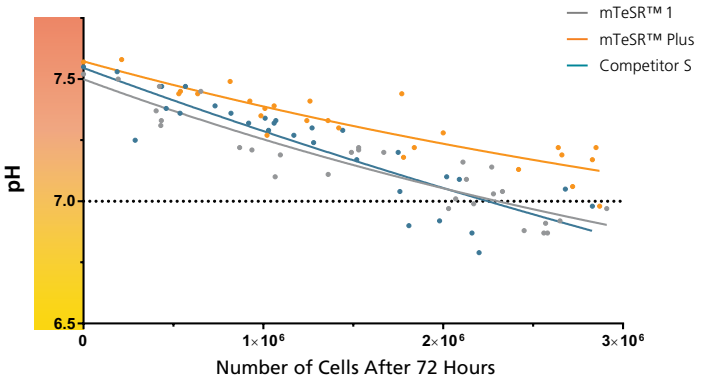


Figure 1. mTeSR™ Plus Maintains Optimal pH Levels Throughout a Weekend-Free Protocol

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. Range of cell numbers shown represent different densities that would be observed throughout a typical passage. This demonstrates that feeds can be skipped for two days at any time during routine maintenance using mTeSR™ Plus while maintaining a pH above 7.0. Note: Cultures were fed double the standard medium volume prior to the 72-hour period without feeds in all media and cell numbers are from one well of a 6-well plate.

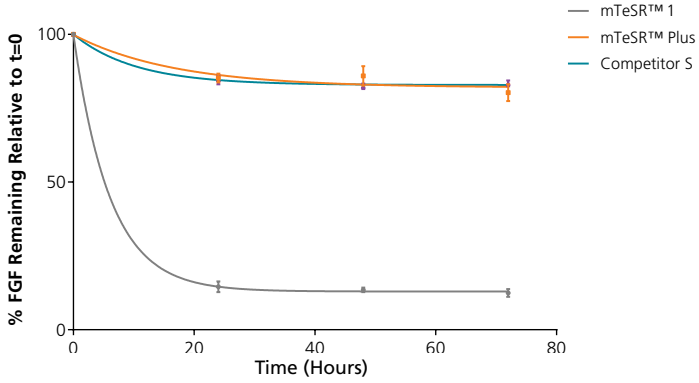


Figure 2. mTeSR™ Plus Maintains Consistent Levels of FGF2 Throughout a Weekend-Free Protocol

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

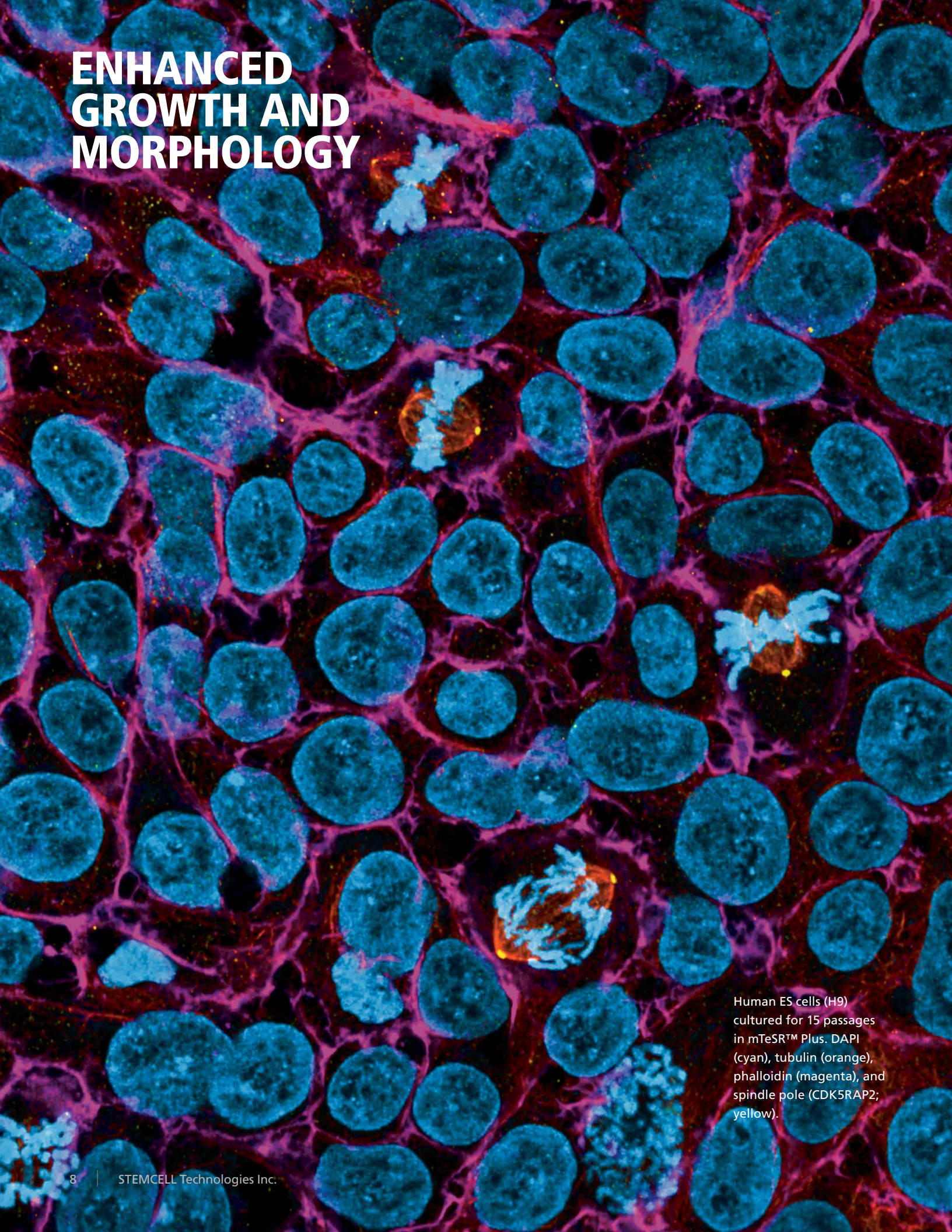
Tested to Work

To test the limits of mTeSR™ Plus and to ensure that the medium upholds cell quality under rigorous standards, all mTeSR™ Plus performance testing was completed using a highly reduced feeding schedule, as shown below.

	MON	TUE	WED	THU	FRI	SAT	SUN
Restricted	P	-	F	-	2F	-	-

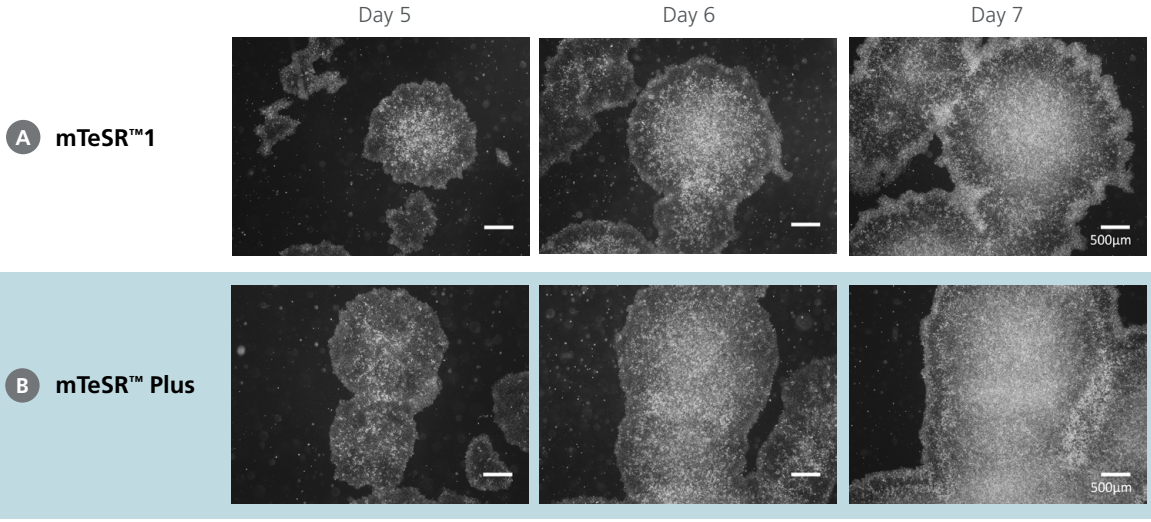
☑

ENHANCED GROWTH AND MORPHOLOGY



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

Superior Morphology of Undifferentiated hPSC Colonies



Uniform, densely packed colonies with defined borders are indicators of high cell quality.

Dense centers that are phase-bright indicate healthy colonies that are ready for passaging.

Figure 3. Human ES Cells in mTeSR™ Plus Display Improved Morphology and Larger Colony Size When Feeding is Restricted

Human ES cells (H9) cultured in (A) mTeSR™1 or (B) mTeSR™ Plus with restricted feeding on Corning® Matrigel®. Images were taken on days 5, 6, and 7 after seeding. PSC colonies grown in mTeSR™ Plus are larger in size and exhibit tighter cellular packing at the edges, resulting in greater border definition compared to mTeSR™1 when feeding is restricted.

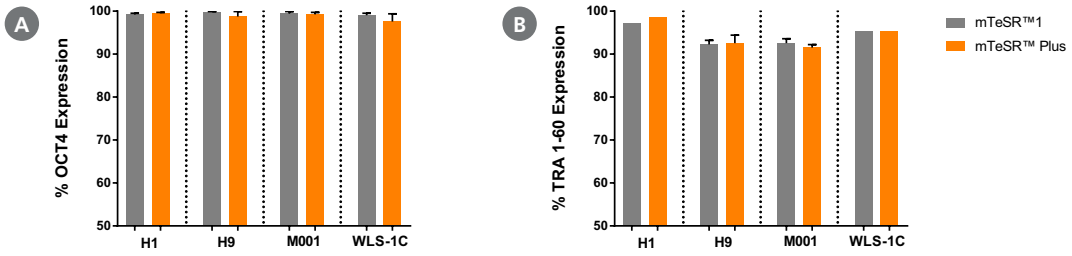


Figure 4. Cells Cultured in mTeSR™ Plus Medium with Restricted Feeding Express Undifferentiated Cell Markers

Human ES (H1, H9) and iPS (WLS-1C, STiPS-M001) cells were characterized using flow cytometry for undifferentiated cell markers, (A) OCT3/4 and (B) TRA-1-60. Graphs show average expression (± SEM) results from analyses of duplicate wells every 5 passages for up to 10-15 passages in mTeSR™1 (daily feeds), or mTeSR™ Plus (restricted feeds).

Faster hPSC Growth Without Altering Cell Division Times

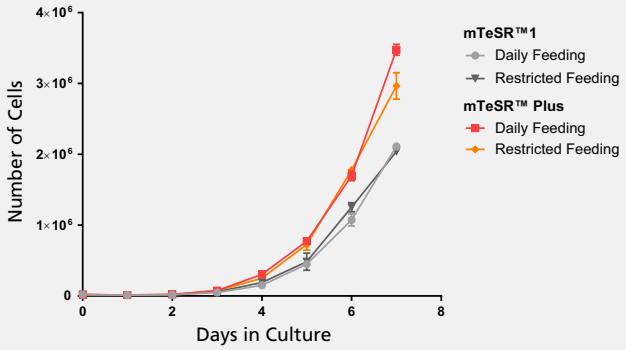


Figure 5. mTeSR™ Plus Supports Higher Cell Numbers

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.

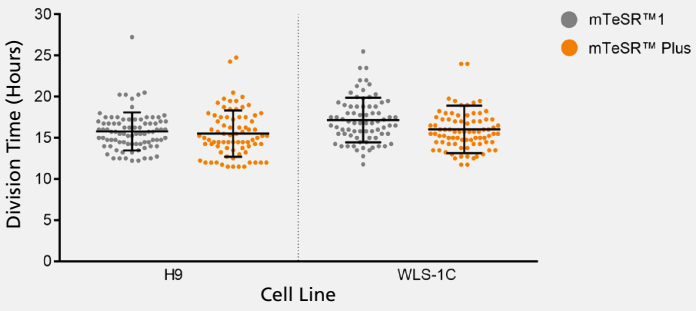


Figure 6. hPSCs Maintained in mTeSR™ Plus Demonstrate Equivalent Cell Division Times to Cells Maintained in mTeSR™1

Human ES and iPS cells (H9, 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.

VALIDATION OF PLURIPOTENCY

Efficient Differentiation Into the Embryonic Germ Layers

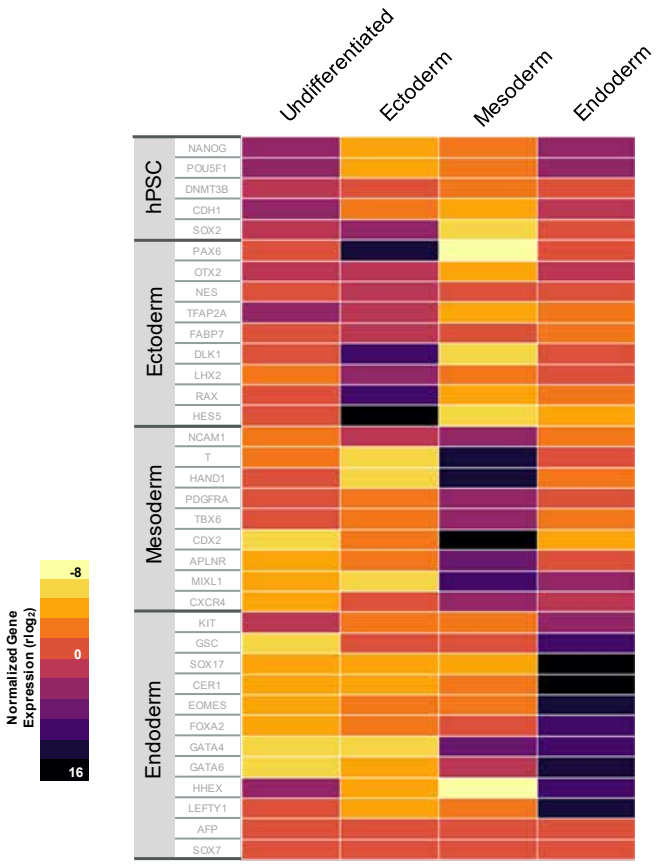


Figure 7. Molecular Analysis of hPSCs Cultured in mTeSR™ Plus with Restricted Feeding Show Efficient Differentiation to All Three Germ Layers

Human iPS (WLS-1C) cells were maintained in mTeSR™ Plus on a restricted feeding schedule and directed to each of the three germ layers using directed differentiation protocols. Undifferentiated cells, differentiated ectoderm, mesoderm, and endoderm cells were then analyzed using the hPSC Trilineage Differentiation qPCR Array to evaluate expression levels of key germ layer markers. Cells cultured with mTeSR™ Plus and differentiated showed clear upregulation of appropriate germ layer-specific markers.

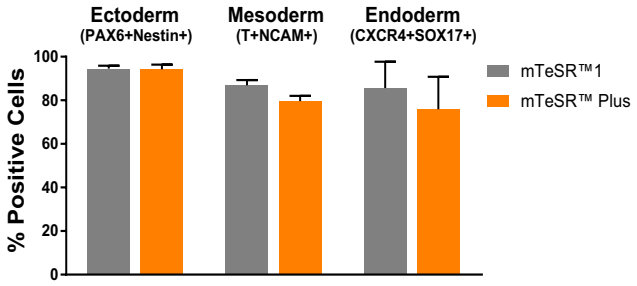


Figure 8. Cells Maintained in mTeSR™ Plus with Restricted Feeding Have Comparable Differentiation Efficiencies to Cells Maintained in mTeSR™1

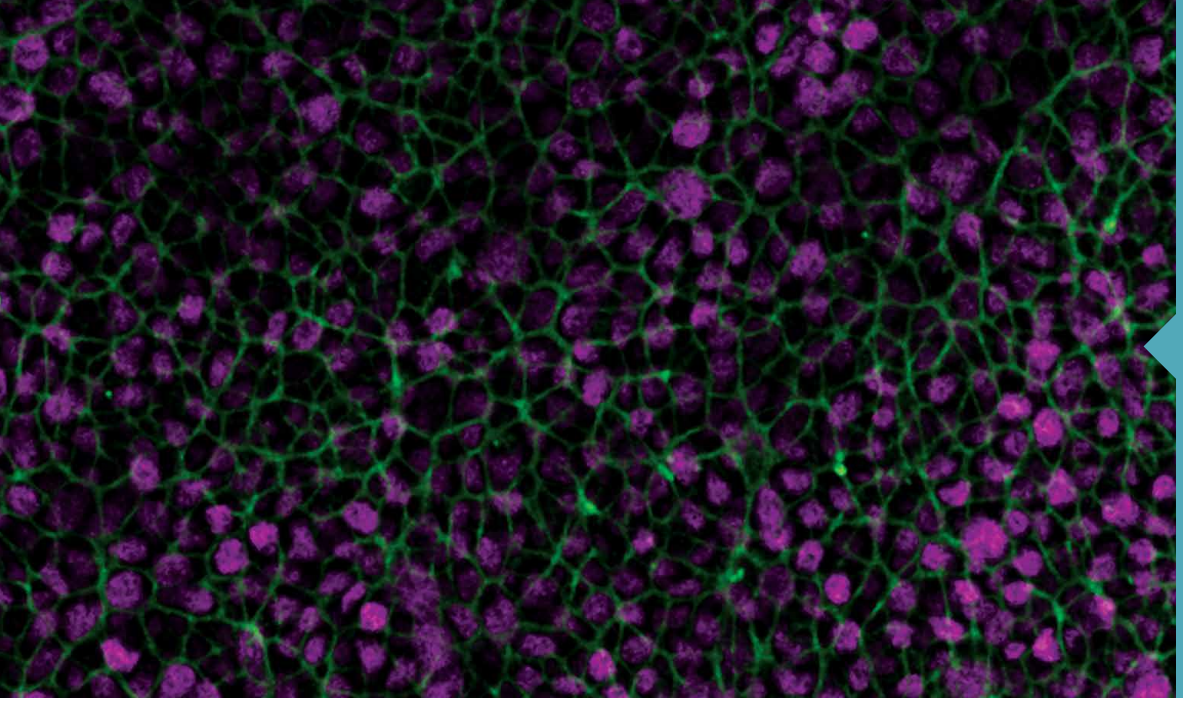
Human ES (H1, H9) and iPS (WLS-1C, STiPS-M001) cells were maintained in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds). Cells were differentiated using directed differentiation protocols and subjected to flow cytometry analysis. Graphs show average expression (± SEM) results from the 4 cell lines. The markers used for flow cytometry for each germ layer are listed in the bar titles.

How is Pluripotency Assessed?

The potency of hPSCs, and their ability to differentiate into the three primary germ layers, can be evaluated using a number of established strategies. While the teratoma assay is known as the "gold standard" technique, it can be lengthy, cost prohibitive, and variable. Another common method, embryoid body differentiation, is also inherently variable. Fortunately, recent advances in monolayer trilineage differentiation protocols have enabled the rapid validation of pluripotency with more ease-of-use and specificity than before.

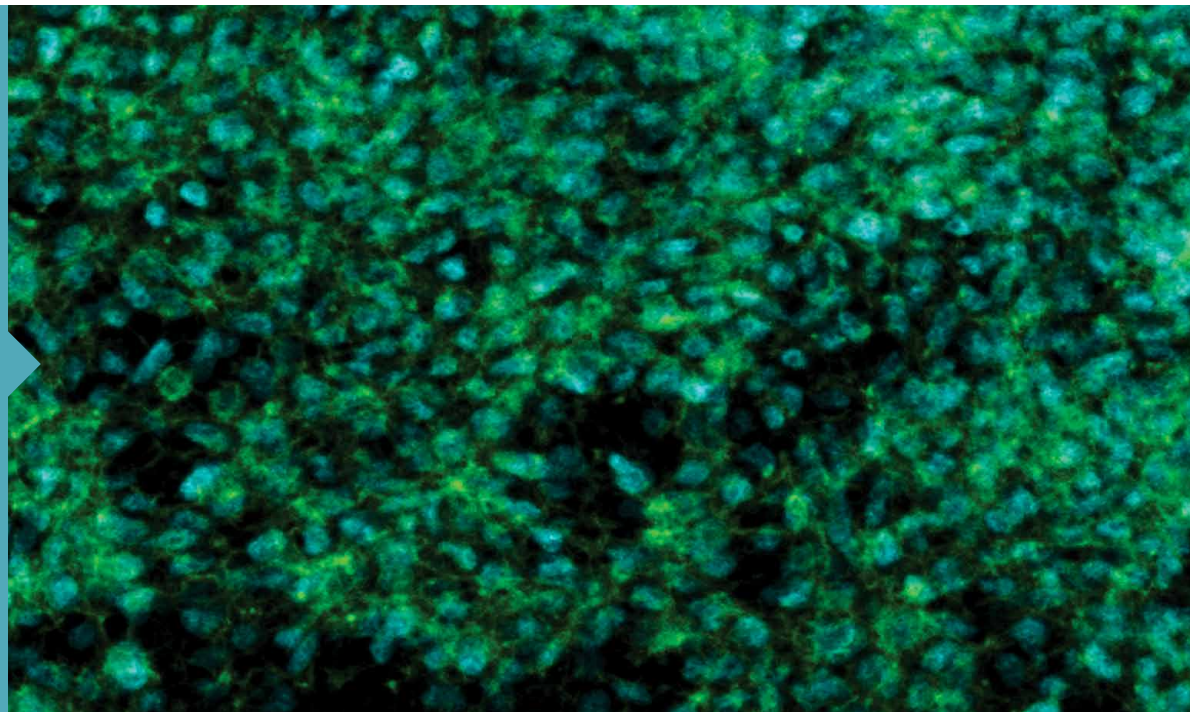
STEMdiff™ Trilineage Differentiation Kit • Format: 1 Kit • Catalog #05230

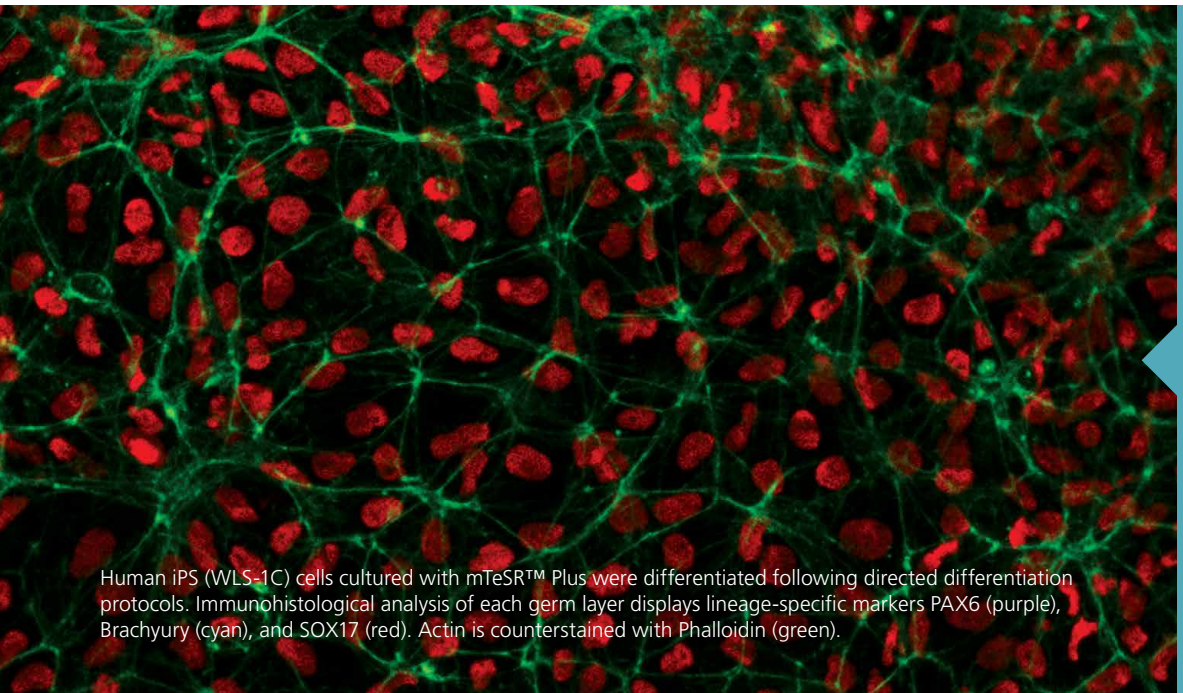
hPSC Trilineage Differentiation qPCR Array • Format: 384-Well Plate • Catalog #07515



ECTODERM

MESODERM

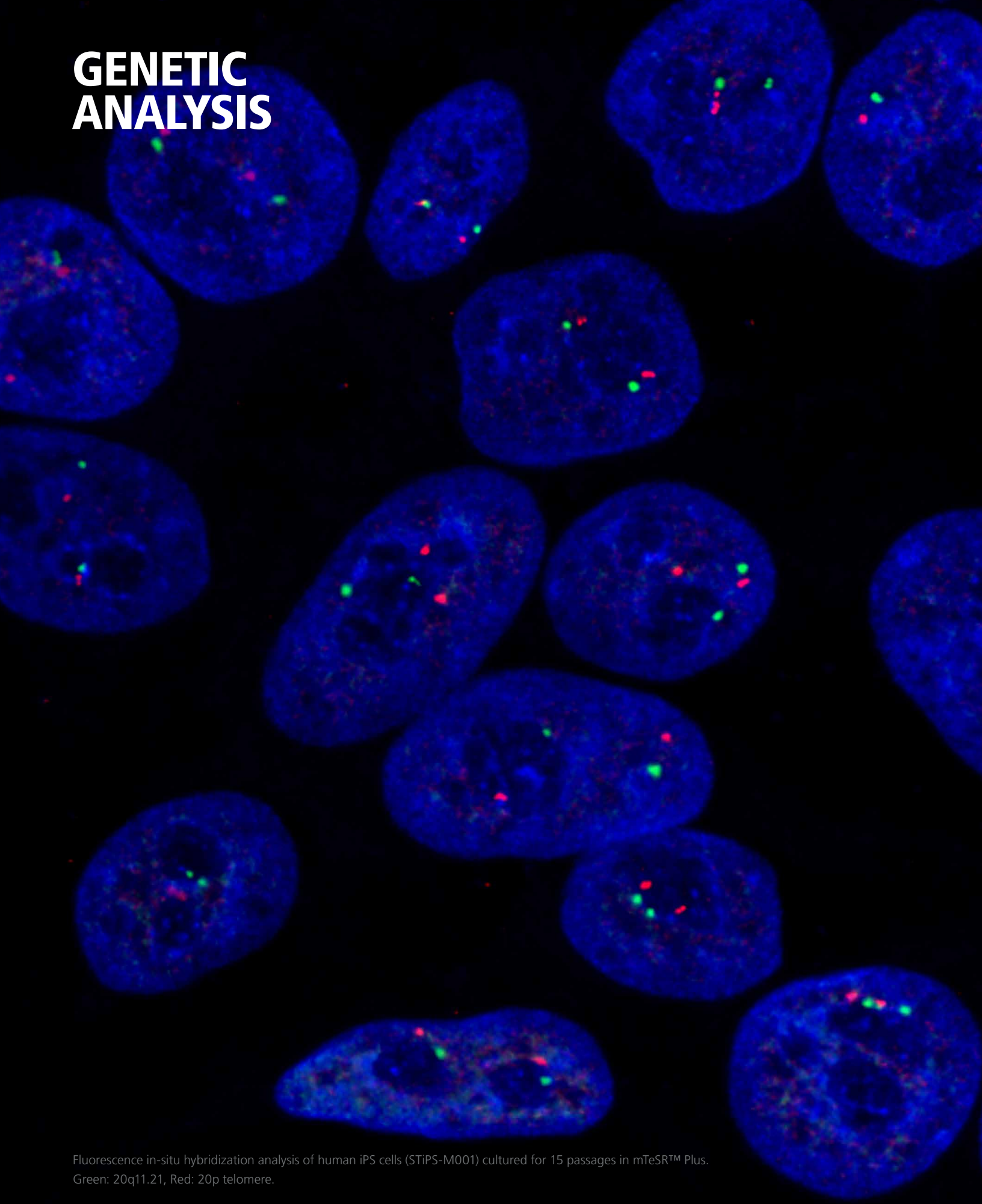




ENDODERM

Human iPS (WLS-1C) cells cultured with mTeSR™ Plus were differentiated following directed differentiation protocols. Immunohistological analysis of each germ layer displays lineage-specific markers PAX6 (purple), Brachyury (cyan), and SOX17 (red). Actin is counterstained with Phalloidin (green).

GENETIC ANALYSIS



Fluorescence in-situ hybridization analysis of human iPS cells (STiPS-M001) cultured for 15 passages in mTeSR™ Plus. Green: 20q11.21, Red: 20p telomere.

Maintenance of Genomic Integrity

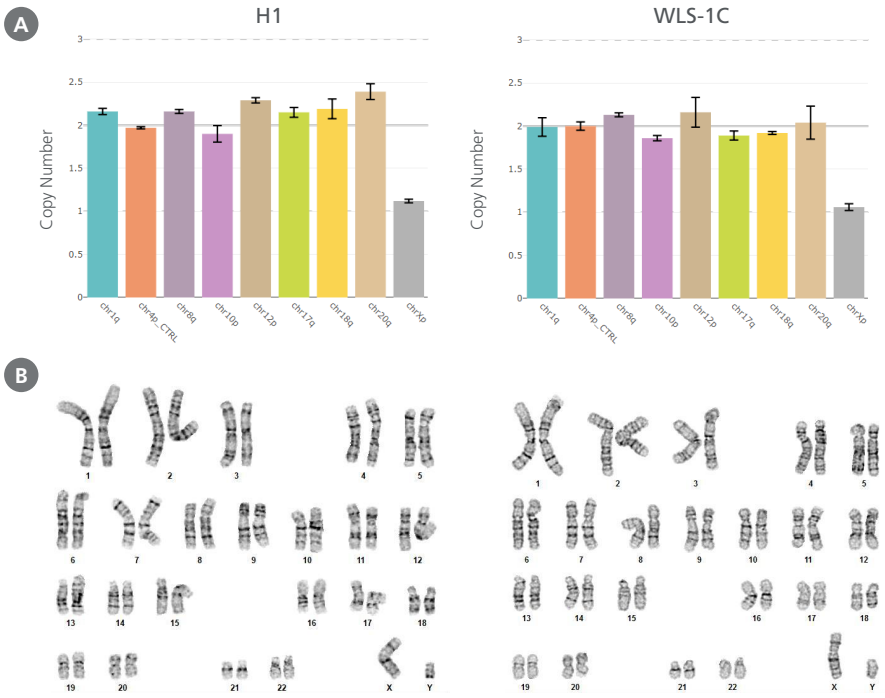


Figure 9. hPSCs Cultured in mTeSR™ Plus with Restricted Feeding Maintain a Normal Karyotype

Human ES (H1) and iPS (WLS-1C) cells were cultured in mTeSR™ Plus for 15 passages. No common abnormalities were detected by the (A) hPSC Genetic Analysis Kit at p5, 10, or 15 (passage 15 shown) and cultures displayed a normal karyotype by (B) G-banding at p30.

Consistent hPSC Gene Expression Profile

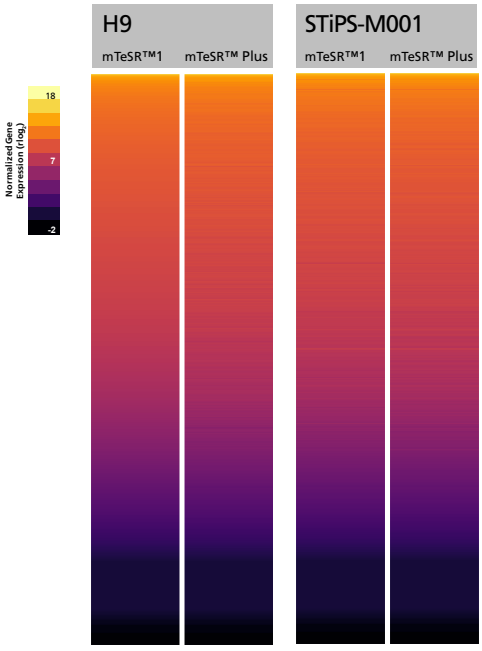


Figure 10. Cells Maintained in mTeSR™ Plus with Restricted Feeding Are Equivalent to Cells Maintained in mTeSR™1 with Daily Feeding

Human ES (H9) and iPS (STiPS-M001) cells were cultured for at least 10 passages with either mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds). Transcriptome analysis of hPSCs maintained in mTeSR™ Plus using RNAseq shows a gene expression profile indistinguishable from cultures maintained in mTeSR™1. Heat map displays all 19,665 genes measured for each condition.

Why Monitor Genetic Stability?

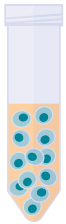
hPSCs have the capacity to acquire karyotypic abnormalities in a non-random and sporadic manner during routine culture. The mechanisms behind these cytogenetic changes remain unknown. A number of these recurrent abnormalities are also observed in human cancers, raising safety concerns over using these cells for therapeutic applications. The most commonly affected chromosomes in hPSCs include 1, 8, 10, 12, 17, 18, 20, and X. Recent studies suggest that acidosis of culture medium may be associated with increased levels of DNA damage, genomic instability, and growth arrest of hPSCs. Due to the stabilization enhancements of mTeSR™ Plus, medium pH is sustained, reducing the rapid onset of acidosis.

hPSC Genetic Analysis Kit • Format: 1 Kit • Catalog #07550

GUIDE TO GENOME EDITING IN mTeSR™ Plus

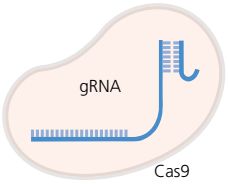
ArciTect™ Cas9 Nuclease • Catalog #76002
ArciTect™ crRNA • Catalog #76010
ArciTect™ tracrRNA Kit • Catalog #76016

Step 1: Culture Cells and Prepare Single-Cell Suspension



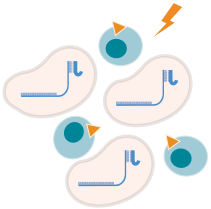
For optimal results, collect cells during exponential growth phase

Step 2: Form Ribonucleoprotein (RNP) Complex



Allow 10-20 minutes for RNP formation

Step 3: Deliver RNP Complex into hPSCs



Electroporate single cell suspension of hPSCs

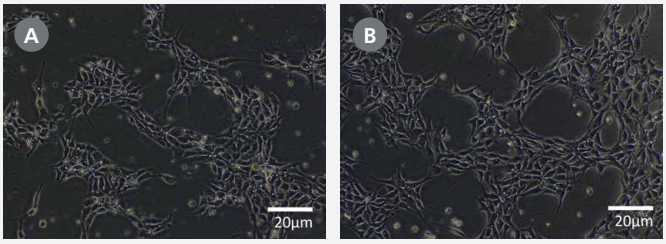


Figure 11. Representative Cell Morphology 24 Hours After RNP Electroporation in mTeSR™1 and mTeSR™ Plus

H1-eGFP ES cells were plated in (A) mTeSR™1 and (B) mTeSR™ Plus and supplemented with CloneR™ immediately following RNP electroporation. Images were taken 24 hours after electroporation.

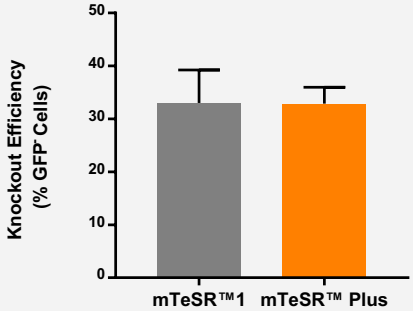


Figure 12. CRISPR-Cas9 Genome Editing Efficiency is Comparable for Cells Cultured in mTeSR™1 with Daily Feeding and mTeSR™ Plus with Restricted Feeding

H1-eGFP ES cells were electroporated with RNP complexes targeting the eGFP transgene (15:30 pmol Cas9:gRNA) and knockout efficiency measured 72 hours following electroporation. Knockout efficiency (± SEM) was measured as % GFP negative (GFP-) cells in test condition subtracted by % GFP- cells in non-electroporated controls. n = 3 biological replicates.

Efficient Genome Editing of hPSCs

The ease-of-use and versatility of CRISPR-Cas9 has been pivotal to furthering our understanding of how individual genes and genetic variants impact biology and disease pathogenesis. Used in combination with stem cells, it has revolutionized the field. For complex cell types like hPSCs, pre- and post-editing culture are critical steps that must be optimized for experimental success during genome editing. mTeSR™ Plus works seamlessly with our ArciTect™ CRISPR-Cas9 system for high-efficiency editing, and, when supplemented with CloneR™, provides a robust culture system to support high levels of post-editing survival for effective clonal derivation and expansion.

CloneR™ • Size: 10 mL • Catalog #05888

Step 4: Recovery/Editing

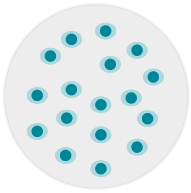
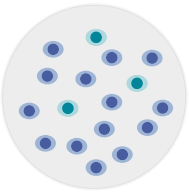


Plate cells into mTeSR™ Plus media supplemented with CloneR™ and allow cells to recover for 48 - 72 hours following RNP delivery.

Step 5: Analysis of Editing Efficiency



Depending on experimental design, editing efficiency can be assessed using the ArciTect™ T7 Endonuclease I Kit, through flow cytometry-based approaches, or by genomic DNA sequencing.

Step 6: Derivation & Characterization of Clonal hPSC Lines

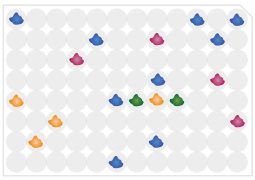


Plate cells at clonal density and use CloneR™ to enhance cloning efficiency. Use editing efficiency calculated in Step 5 to estimate the approximate number of clones to select for further characterization.

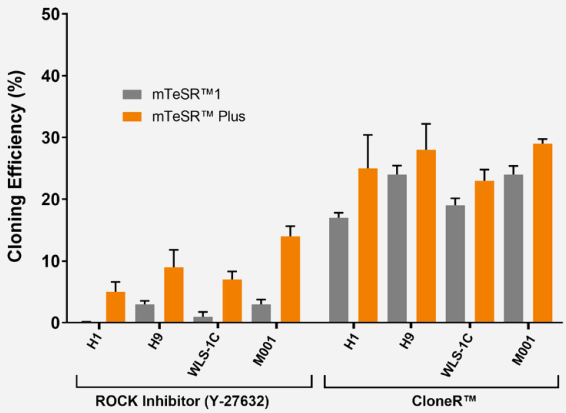


Figure 13. High Cloning Efficiency of hPSCs in mTeSR™ Plus Supplemented with CloneR™

hPSCs (H1, H9, WLS-1C, and STiPS-M001) plated in mTeSR™ Plus with CloneR™ demonstrate cloning efficiencies equal to or greater than hPSCs in mTeSR™1 with CloneR™. Cells were seeded at clonal density (25 cells/cm²) in mTeSR™1 or mTeSR™ Plus on CellAdhere™ Vitronectin™ XF™-coated plates. n ≥ 3 biological replicates.

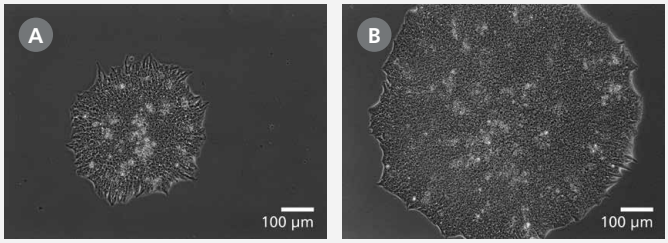
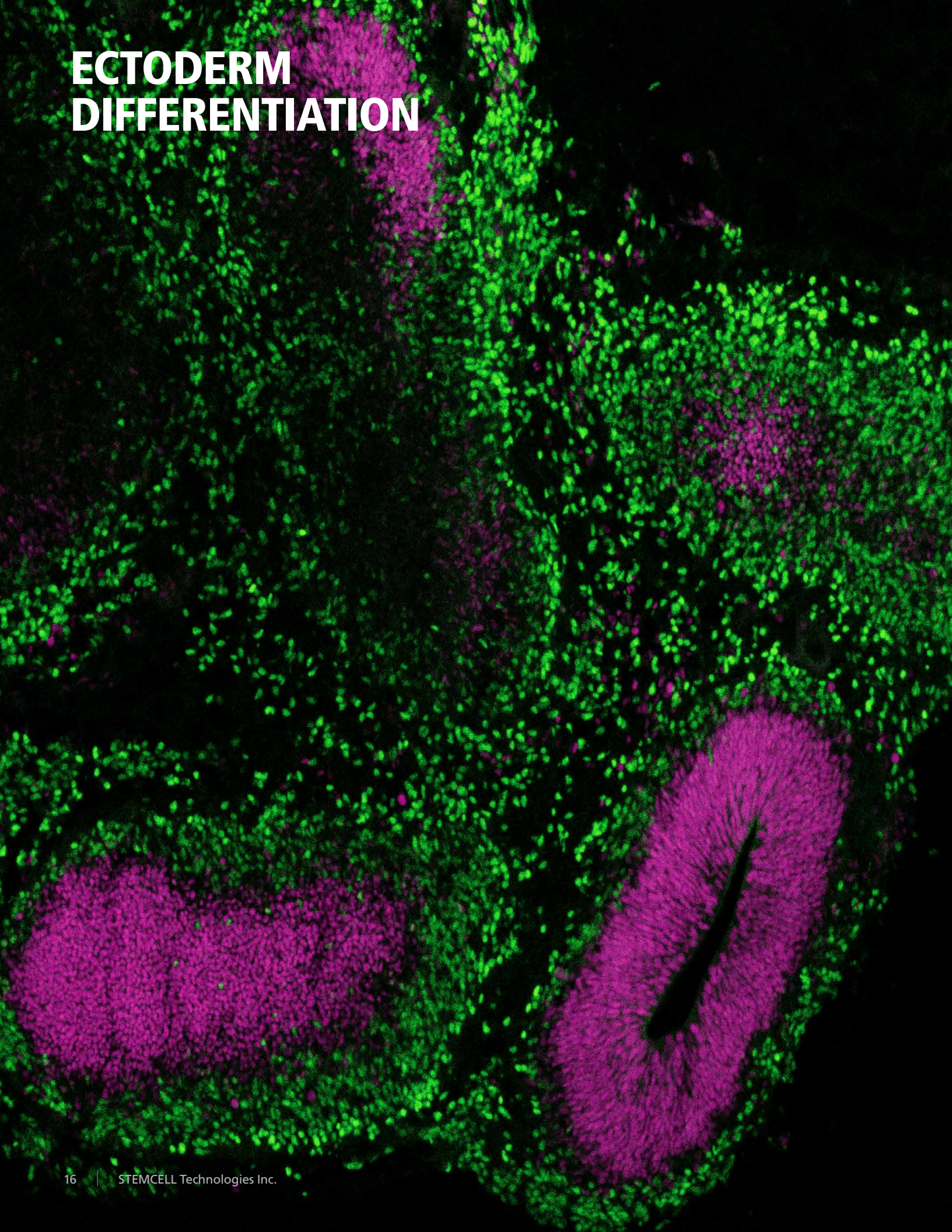


Figure 14. Clones Derived in mTeSR™ Plus are Larger and Ready to Be Picked at an Earlier Timepoint

Representative images of human ES (H9) colonies taken 8 days following single-cell plating at clonal density (25 cells/cm²) in either (A) mTeSR™1 or (B) mTeSR™ Plus supplemented with CloneR™ on CellAdhere™ Vitronectin™ XF™-coated plates.

ECTODERM DIFFERENTIATION



Neural Progenitor Cells

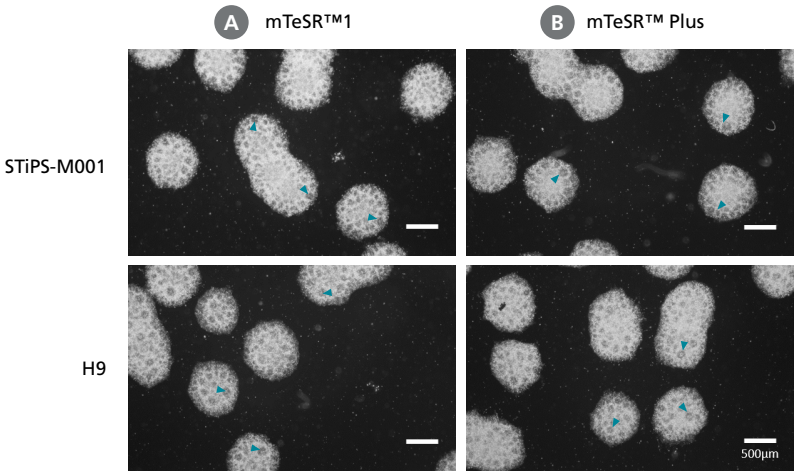


Figure 15. Generation of Neural Progenitor Cells from hPSCs Maintained in mTeSR™ Plus

Human ES (H9) and iPS (STiPS-M001) cells were maintained in (A) mTeSR™1 with daily feeds or (B) mTeSR™ Plus with restricted feeds and differentiated using an embryoid body (EB)-based protocol with STEMdiff™ SMADi Neural Induction Kit. Neural progenitor cells derived from hPSCs maintained in either mTeSR™1 or mTeSR™ Plus clearly display neural rosettes (arrowheads) after replating EBs.

STEMdiff™ SMADi Neural Induction Kit • Format: 1 Kit • Catalog #08581

Astrocytes

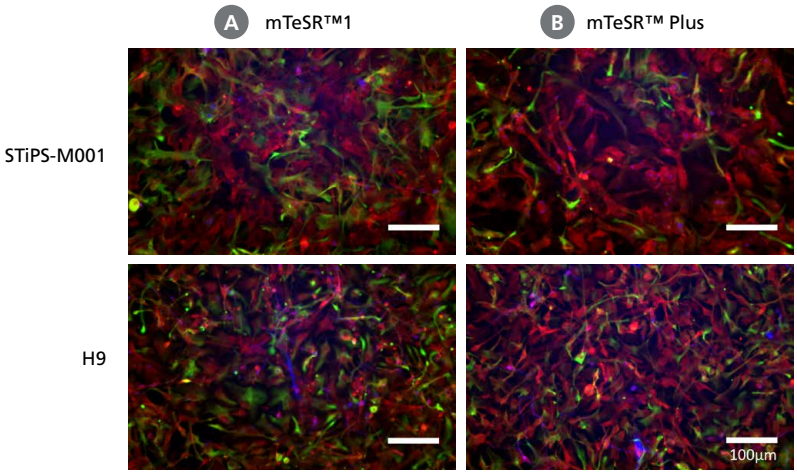


Figure 16. Downstream Astrocyte Differentiation of Neural Progenitor Cells Derived from hPSCs Maintained in mTeSR™ Plus

Neural progenitor cells generated from human ES (H9) and iPS (STiPS-M001) cells maintained in (A) mTeSR™1 with daily feeds or (B) mTeSR™ Plus with restricted feeds were differentiated and matured to astrocytes using the STEMdiff™ Astrocyte Differentiation Kit for 25 - 26 days and STEMdiff™ Astrocyte Maturation Kit for 18 days. The astrocytes express GFAP (green), S100B (red), and DCX (blue).

STEMdiff™ Astrocyte Differentiation Kit • Format: 1 Kit • Catalog #08540

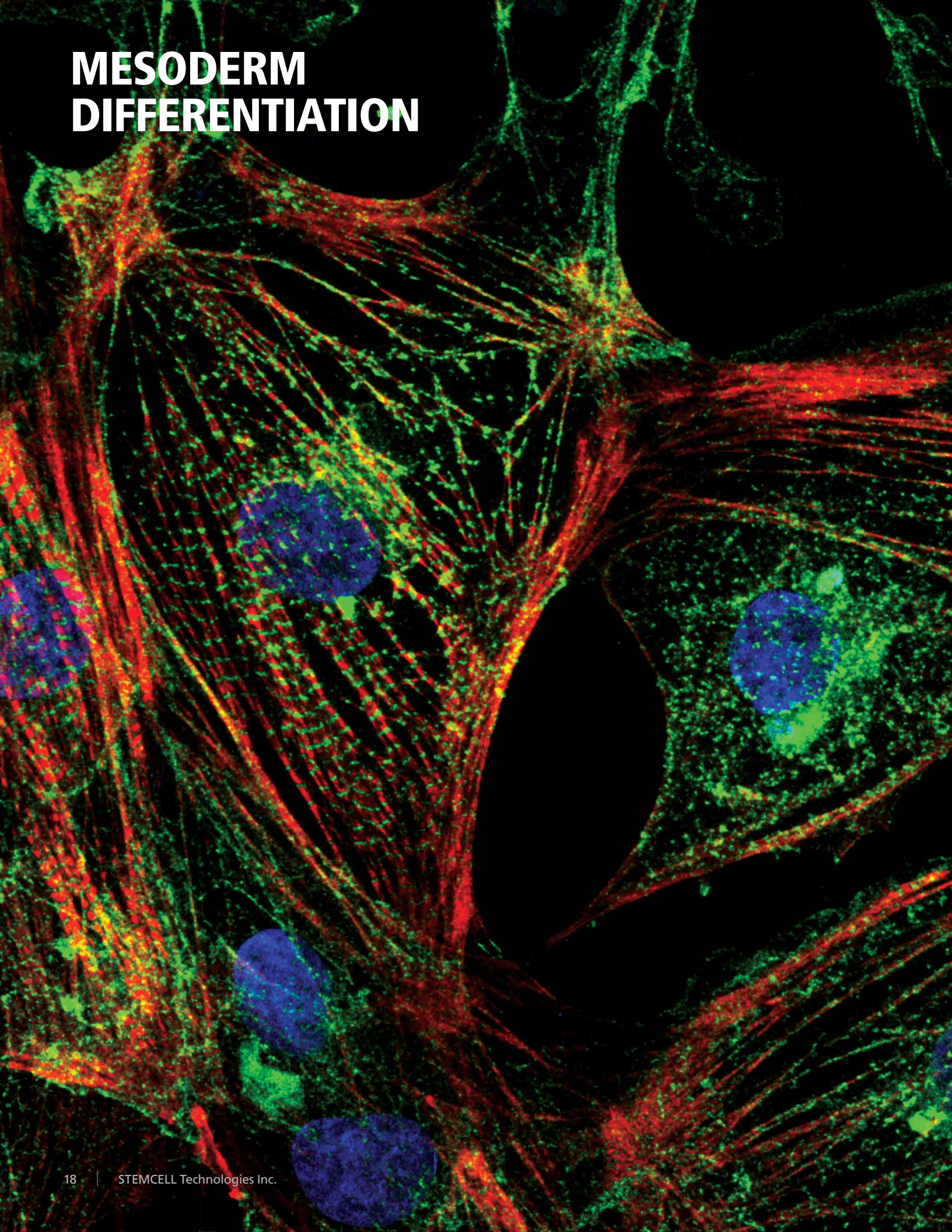
STEMdiff™ Astrocyte Maturation Kit • Format: 1 Kit • Catalog #08550

Cerebral Organoids

Human ES (H9) cells were cultured with mTeSR™ Plus and directed to cerebral organoids using the STEMdiff™ Cerebral Organoid Kit. Image shows apical progenitor marker SOX2 (purple) and neuronal marker TBR1 (green).

STEMdiff™ Cerebral Organoid Kit • Format: 1 Kit • Catalog #08570

MESODERM DIFFERENTIATION



Hematopoietic Progenitor Cells

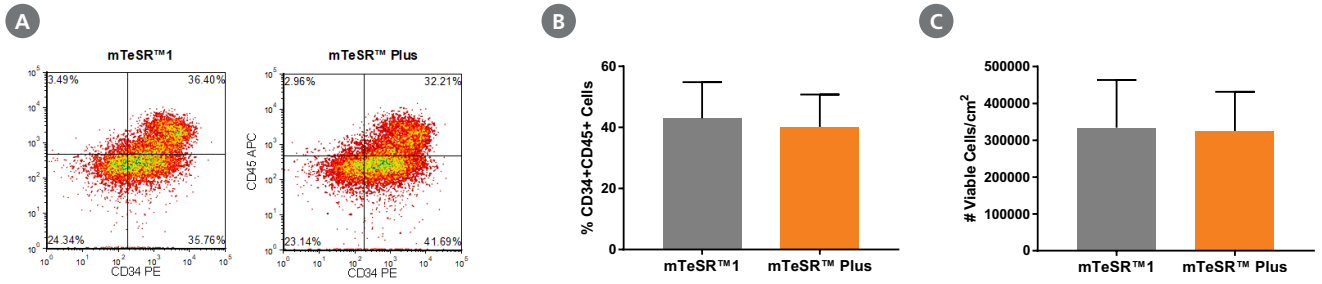


Figure 17. Generation of Hematopoietic Progenitor Cells from hPSCs Maintained in mTeSR™ Plus

Human ES (H1, H9) and iPS (STiPS-M001, WLS-1C) cell lines maintained in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds) were differentiated to hematopoietic progenitor cells using the STEMdiff™ Hematopoietic Kit. At the end of the differentiation period, cells were harvested from the supernatant and analyzed by flow cytometry for co-expression of $CD34^+$ and $CD45^+$. (A) Representative density plots showing $CD34^+$ and $CD45^+$ expression, (B) percentage of cells co-expressing $CD34^+$ and $CD45^+$, and (C) total number of viable cells harvested are shown. Data are expressed as the mean (± SEM); n=4.

STEMdiff™ Hematopoietic Kit • Format: 1 Kit • Catalog #05310

Cardiomyocytes

Human iPS (STiPS-M001) cells maintained with mTeSR™ Plus were directed to cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit. Image shows cardiomyocyte markers cardiac troponin T (cTNT; red) and alpha-actinin (green). Nuclei are counterstained with DAPI (blue).

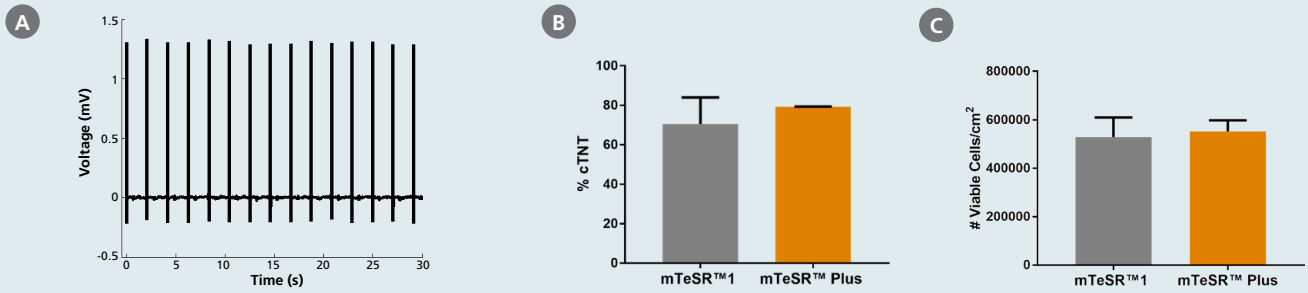
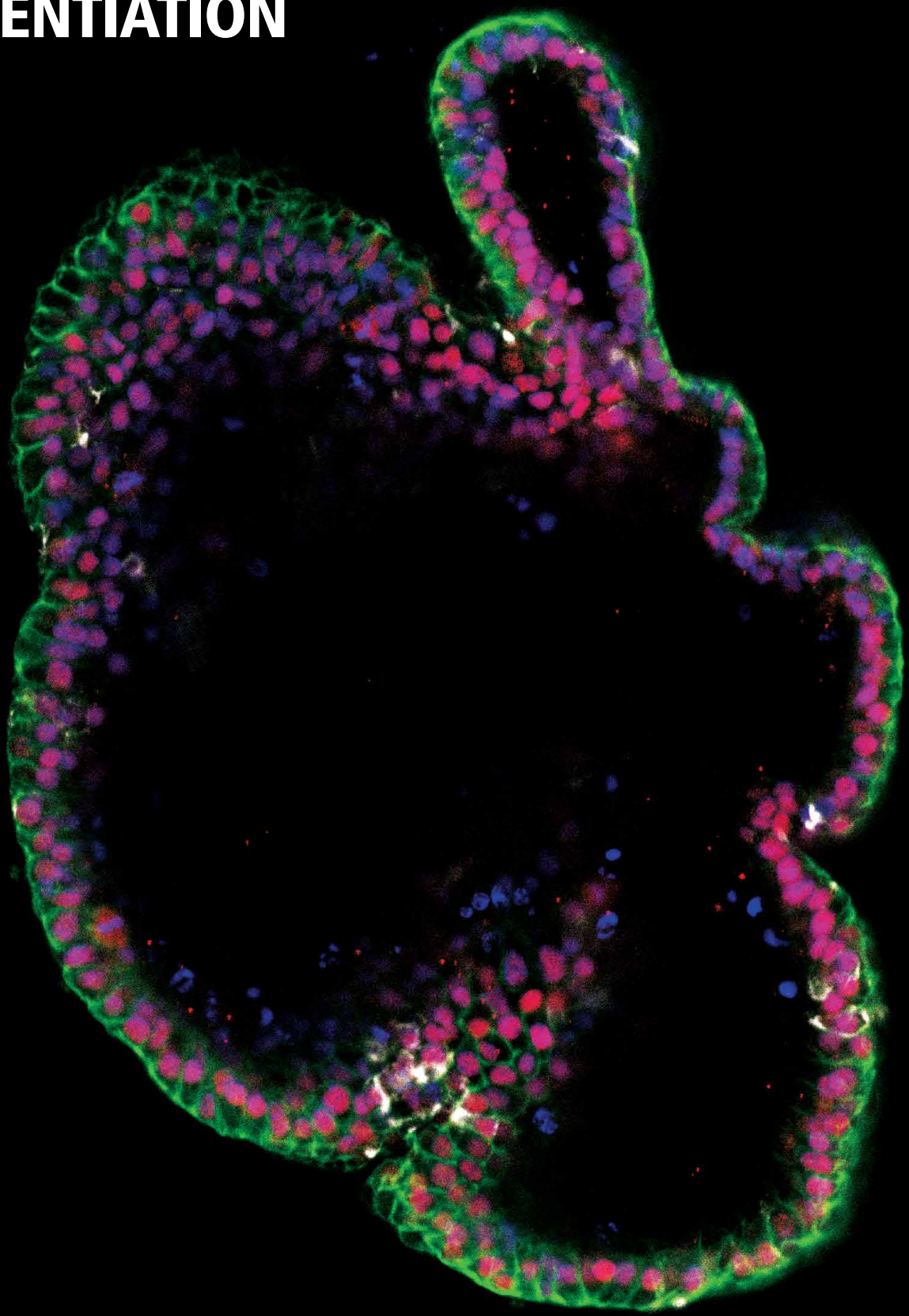


Figure 18. Generation of Cardiomyocytes from hPSCs Maintained in mTeSR™ Plus

Human ES (H9) and iPS (WLS-1C) cells were maintained in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds) and differentiated to cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit. At the end of the differentiation period, cells were harvested and analyzed by microelectrode array (MEA) and flow cytometry. (A) Representative MEA voltage recordings of cardiomyocytes (day 20) demonstrate a characteristic electrical profile and stable beat rate. (B) Percentages of cells expressing cTNT and (C) total number of viable cells harvested are shown. Data are expressed as the mean (± SEM); n=2.

STEMdiff™ Cardiomyocyte Kit • Format: 1 Kit • Catalog #05120

ENDODERM DIFFERENTIATION



Definitive Endoderm

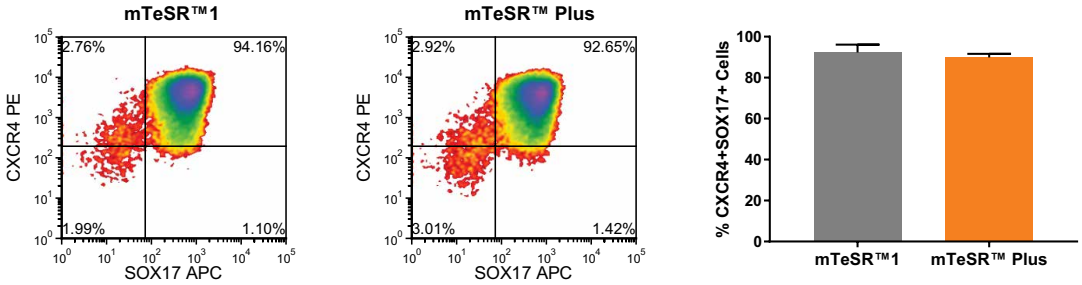


Figure 19. Generation of Definitive Endoderm from hPSCs Maintained in mTeSR™ Plus

(A) Representative density plots showing CXCR4 and SOX17 expression in cells cultured in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds), following 5 days of differentiation using the STEMdiff™ Definitive Endoderm Kit. (B) Quantitative analysis of definitive endoderm formation in multiple hPSC lines (H9, STiPS-M001, WLS-1C) maintained with mTeSR™1 or mTeSR™ Plus as measured by co-expression of CXCR4 and SOX17. Data are expressed as the mean percentage of cells (± SEM) expressing both markers; n=3.

STEMdiff™ Definitive Endoderm Kit • Format: 1 Kit • Catalog #05110

Pancreatic Progenitor Cells

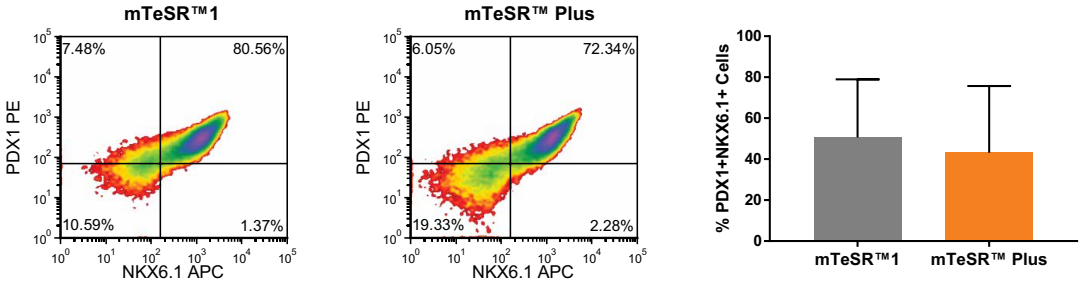


Figure 20. Generation of Pancreatic Progenitors from hPSCs Maintained in mTeSR™ Plus

(A) Representative density plots showing PDX-1 and NKX6.1 expression in cells cultured in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds), following differentiation using the STEMdiff™ Pancreatic Progenitor Kit. (B) Quantitative analysis of pancreatic progenitor formation in multiple hPS (H9, STiPS-M001, WLS-1C) cell lines maintained with mTeSR™1 or mTeSR™ Plus as measured by co-expression of PDX-1 and NKX6.1. Data are expressed as the mean percentage of cells (± SEM) expressing both markers; n=3.

STEMdiff™ Pancreatic Progenitor Kit • Format: 1 Kit • Catalog #05120

Intestinal Organoids

Human ES (H9) cells were cultured with mTeSR™ Plus and directed to intestinal organoids using the STEMdiff™ Intestinal Organoid Kit. Image shows markers of the intestinal epithelium EpCAM (green) and CDX2 (red), and intestinal mesenchyme marker vimentin (white). Nuclei are counterstained with DAPI (blue).

STEMdiff™ Intestinal Organoid Kit • Format: 1 Kit • Catalog #05140

FREQUENTLY ASKED QUESTIONS

Q: How do I transition my cell lines into mTeSR™ Plus?

A: Cells cultured in feeder-free media can be conveniently transitioned into mTeSR™ Plus without any adaptation step. We routinely transition cell lines maintained in mTesR™1 on Matrigel® directly into mTeSR™ Plus on Matrigel® and cells do not demonstrate a lag period following the first passage. Often, an enhanced growth rate is observed immediately, resulting in larger colonies and a higher confluency sooner after passaging.

Q: Do I still need to feed my cells every day?

A: You do not! To skip a single day (48 hours), we recommend performing a "single feed" as usual (for example, 2 mL per well of a 6-well plate). To skip two consecutive days (72 hours), perform a "double feed" (for example, 4 mL per well of a 6-well plate). Of course, cultures can still be fed every day with mTeSR™ Plus—it's all up to your desired schedule. To see some examples of feeding schedules, please refer to page 7.

Q: Do cell lines behave differently in mTeSR™ Plus?

A: Population doubling time is reduced in mTeSR™ Plus compared with mTeSR™1. Colonies appear larger and more dense, with well-defined edges. As a result, cell seeding densities or passaging intervals may need to be adjusted. To keep the same passaging interval, we recommend seeding ~20 - 25% fewer clumps at passage than would normally be seeded into mTeSR™1. You may wish to test a number of split ratios to determine which best suits your specific method of hPSC maintenance. For more detailed guidance, feel free to contact us.

Q: Do I need to change how I use my passaging reagent?

A: In some cases, the passaging reagent may need to be exposed to cells for a longer period of time in order to successfully dissociate the larger colonies grown in mTeSR™ Plus. We recommend observing your cells under the microscope to ensure that colonies have dissociated sufficiently prior to scraping or spraying.

Q: Can I thaw cells directly into mTeSR™ Plus?

A: Cryopreserved hPSCs previously maintained in mTeSR™1 can be thawed directly into mTeSR™ Plus with no additional adaptation step. In fact, you are likely to see enhanced recovery rates of cryopreserved hPSCs thawed directly into mTeSR™ Plus.

Q: How do I passage hPSCs as single cells using mTeSR™ Plus?

Extensive passaging of hPSCs as single cells is not recommended in any maintenance medium due to the increased risk of obtaining karyotypic abnormalities using this method. If single-cell passaging is required, we recommend using CellAdhere™ Laminin-521 cell culture matrix (Catalog #77003). If using a matrix other than CellAdhere™ Laminin-521, the addition of CloneR™ (Catalog #05888) or other survival agents such as Y-27632 (Catalog #72302) is advised.

In order to obtain a highly viable population of single cells free of aggregates, incubate with enzymatic dissociation reagent (e.g. Accutase™ Cell Detachment Solution [Catalog #07920]) at 37° for ~5 minutes. We recommend passaging cells when they reach 80 - 90% confluency.

Recommended cell seeding densities:

3 - 4 day passaging = 25,000 cells/cm^2

4 - 5 day passaging = 12,500 cells/cm^2

This protocol may need to be optimized for specific cell lines. Please contact us if you would like a more detailed protocol.

Q: How do I derive clonal hPSC lines in mTeSR™ Plus?

CloneR™ supplement (Catalog #05888) is formulated to significantly increase cloning efficiency of hPSCs. CloneR™ is recommended for use with mTeSR™ Plus when seeding cells at a low density. If seeding multiple cells per well to derive clones, we recommend plating at a maximum density of 25 cells/cm^2 to prevent generation of mosaic colonies. We advise withdrawing CloneR™ from mTeSR™ Plus after 4 days of exposure, as this duration is sufficient for robust clone development. For a complete protocol detailing clonal hPSC line derivation, please refer to the CloneR™ Product Information Sheet, available at www.stemcell.com/cloner.

Q: Will mTeSR™ Plus be available as cGMP?

mTeSR™ Plus has been prioritized in our GMP manufacturing pipeline and will be produced under a cGMP quality management system compliant to CFR 820 in the near future. Please look out for a notification regarding this change towards the end of 2019. Until that point, mTeSR™ Plus will be manufactured under a quality management system certified to ISO 13485, Medical Devices.

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1. Thomson JA et al. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391): 1145-7.
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5. Takahashi K et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5): 861-72.
6. Chen G et al. (2011) Chemically defined conditions for human iPS cell derivation and culture. Nat Methods 8(5): 424-9.
7. Ran FA et al. (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8(11): 2281-308.

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