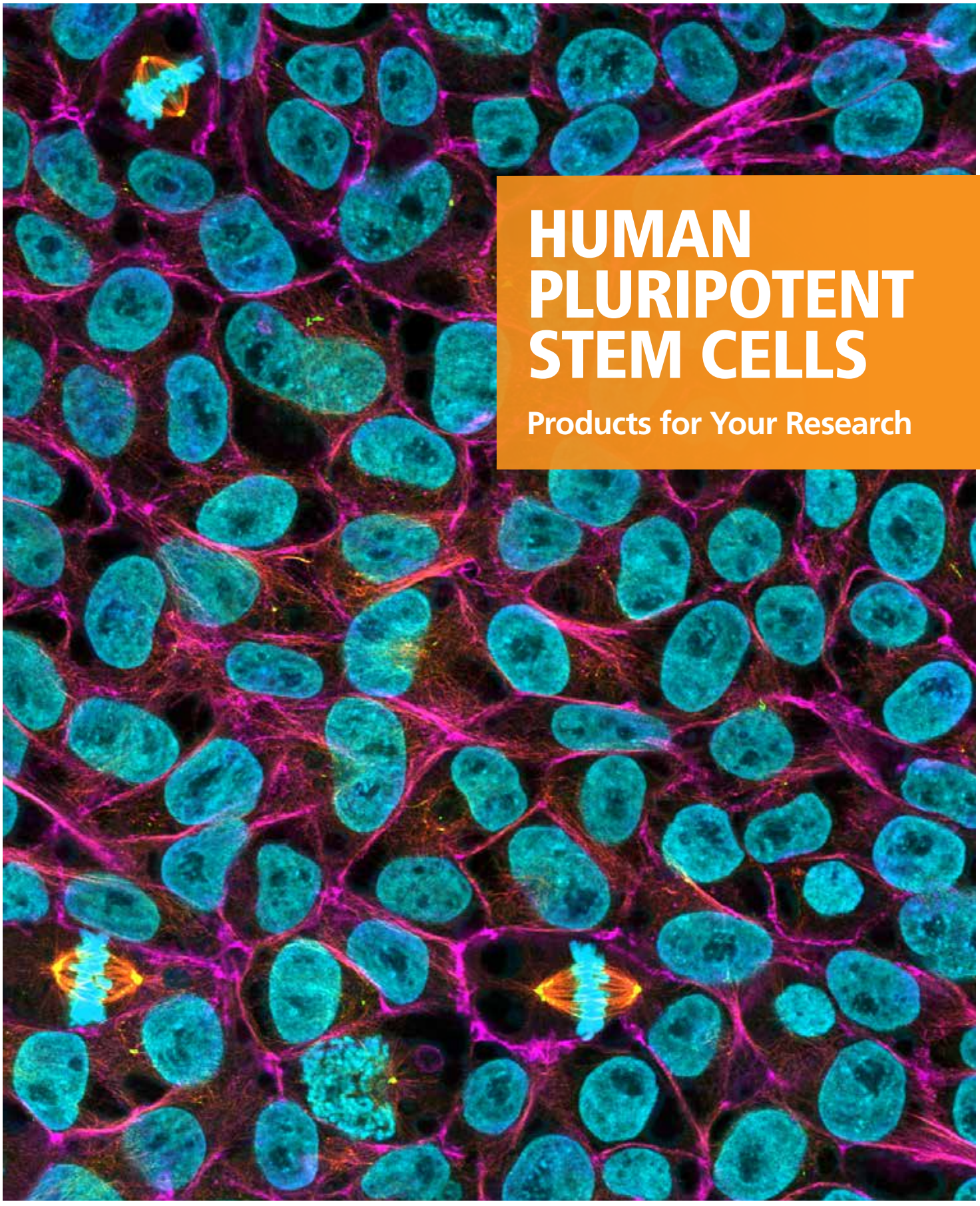

HUMAN PLURIPOTENT STEM CELLS

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Reprogramming

ReproRNA™-OKSGM

Non-Integrating Reprogramming Vector

ReproRNA™-OKSGM 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 (Figure 1). When used together with ReproTeSR™ reprogramming medium, the generation of iPS cell colonies can be achieved under feeder-free conditions with superior colony morphology and similar reprogramming efficiency to feeder-based systems (Figure 2). 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 TeSR™ maintenance media (mTeSR™1, TeSR™2 or TeSR™-E8™) and further differentiated into cells of all three germ layers.

PRODUCT	SIZE	CATALOG #
ReproRNA™-OKSGM Kit*	1 Kit	05930
ReproRNA™-OKSGM	12 µg	05931

* Kit includes the ReproRNA™-OKSGM vector, ReproRNA™ Transfection Reagent, ReproRNA™ Transfection Supplement and Recombinant B18R Protein.

Why Use ReproRNA™-OKSGM?

NON-VIRAL. Non-integrating vector system.

SELF-REPLICATING VECTOR. Only a single transfection is required.

ALL-IN-ONE. Vector contains all reprogramming factors.

HIGHLY EFFICIENT. Comparable fibroblast reprogramming efficiency to Sendai virus.[1]

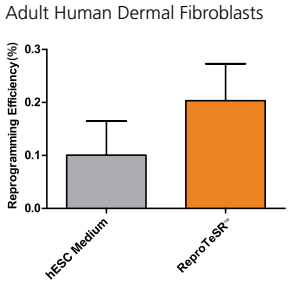


Figure 2. ReproRNA™-OKSGM Vector Efficiently Reprograms Fibroblasts

Human dermal fibroblasts were transfected with the ReproRNA™-OKSGM vector and reprogrammed under feeder-dependent (standard KOSR-containing human embryonic stem (ES) cell medium on inactivated mouse embryonic fibroblasts (iMEFs)) or feeder-free conditions (ReproTeSR™ on Corning® Matrigel®). Fibroblasts (passage 4) were reprogrammed with average efficiencies of 0.10 ± 0.06% (human ES cell medium) and 0.20 ± 0.07% (ReproTeSR™). Reprogramming efficiency of fibroblasts with ReproRNA™ and ReproTeSR™ is comparable to that reported with Sendai virus.[1] (n ≥ 6; data shown are mean ± SD)

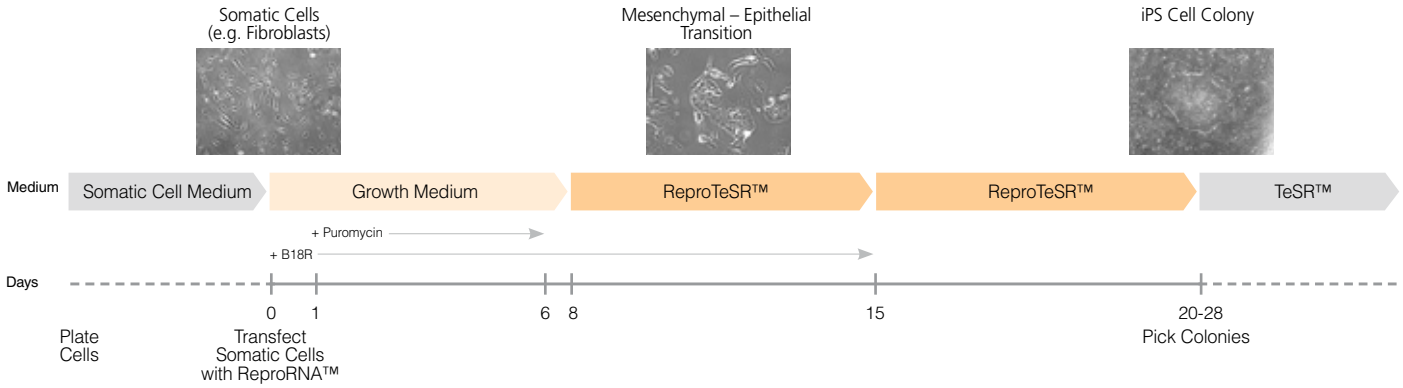


Figure 1. Timeline for Reprogramming with ReproRNA™-OKSGM

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 TeSR™ medium (mTeSR™1, TeSR™2 or TeSR™-E8™).

ReproTeSR™

Reproducible Generation of Human iPS Cells

ReproTeSR™ is a complete, defined, xeno-free and feeder-free reprogramming medium optimized for the generation of human iPS cells. ReproTeSR™ is used during the induction phase of reprogramming (Figure 3) and produces 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 isolation and expansion of hematopoietic cells. ReproTeSR™ can be purchased individually or as part of the Erythroid or CD34$^+$ Progenitor Reprogramming Kits. ReproTeSR™ can also be used to reprogram other somatic cell types, and can be paired with ReproRNA™ for reprogramming fibroblasts. iPS cells generated with ReproTeSR™ can be subsequently cultured with TeSR™ media and differentiated with the STEMdiff™ suite of products to cells of all three lineages.

PRODUCT	SIZE	CATALOG #
ReproTeSR™	500 mL	05920
Erythroid Progenitor Reprogramming Kit	For reprogramming 10 mL of blood	05924
CD34$^+$ Progenitor Reprogramming Kit	For reprogramming 80 mL of blood	05925

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.

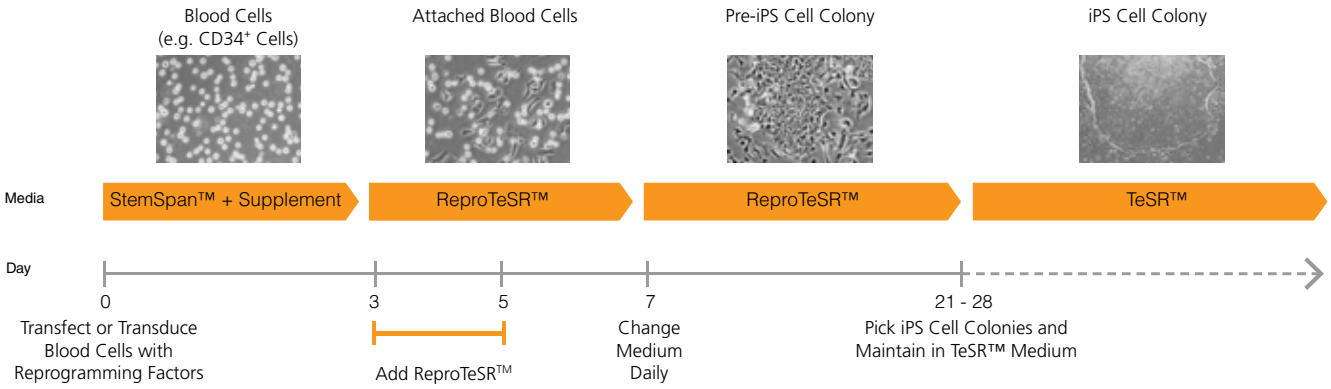


Figure 3. Schematic of ReproTeSR™ Blood Reprogramming Timeline

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 media changes until putative iPS cell colonies emerge (days 21 to 28). iPS cell colonies can then be isolated and propagated in TeSR™ medium (mTeSR™1, TeSR™2 or TeSR™-E8™).

TeSR™-E7™

Animal Component-Free Generation of Human iPS Cells from Fibroblasts

TeSR™-E7™ is a defined, animal component-free (ACF) reprogramming culture medium optimized for the generation of human iPS cells without the use of feeders (Figure 5). It is based on the E7 formulation published by the laboratory of Dr. James Thomson[2] (University of Wisconsin-Madison). TeSR™-E7™ is specifically formulated to limit fibroblast overgrowth, resulting in colonies with easily recognizable ES cell-like morphology (Figure 4).

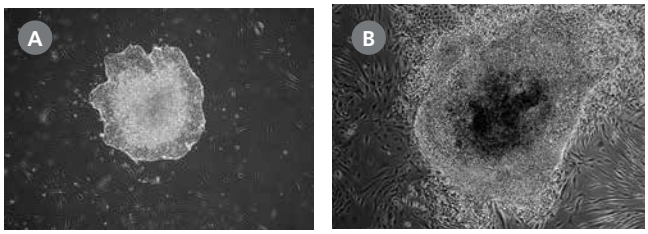


Figure 4. Comparison of Primary iPS Cell Colonies Derived Using TeSR™-E7™ with Qualified versus Unqualified bFGF

(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.

Why Use TeSR™-E7™?

EASY TO IDENTIFY AND SELECT COLONIES. Pre-screened components ensure high-quality colony morphology for improved manual selection.

RAPID SUBCLONING. Reduced differentiation and fibroblast growth enables rapid establishment of homogeneous human iPS cell cultures.

REPRODUCIBLE EFFICIENCY. Feeder-free, defined formulation facilitates reproducibly efficient human iPS cell generation.

PRODUCT	SIZE	CATALOG #
TeSR™-E7™	500 mL	05914

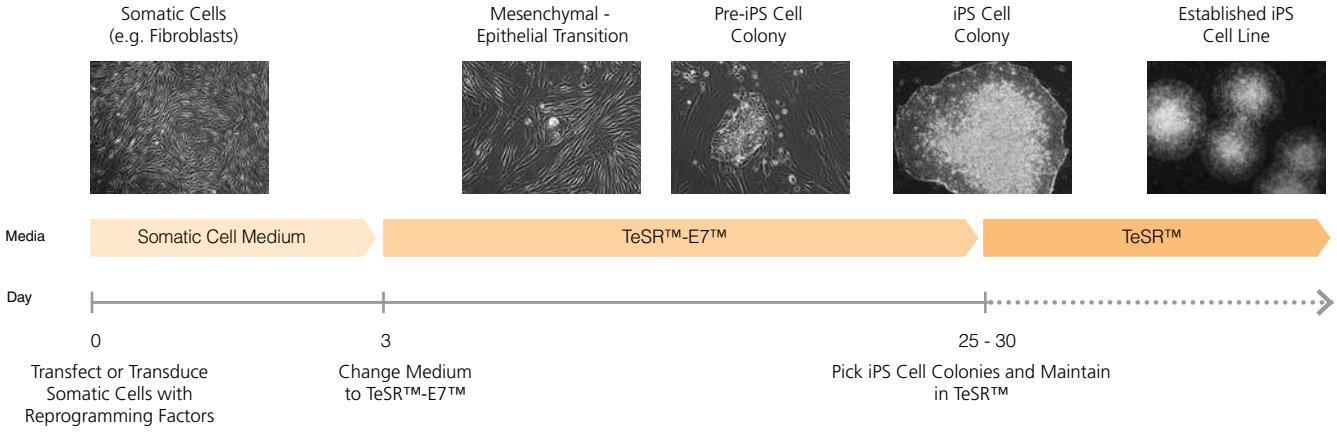


Figure 5. Schematic of Reprogramming Timeline

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 media (mTeSR™1, TeSR™2 or TeSR™-E8™)

Maintenance

Maximize Your Pluripotential with the TeSR™ Family of hPSC Culture Media

Maintenance of high-quality human pluripotent stem cells (hPSCs) is critical to success in all applications of hPSC research. The TeSR™ family of feeder-free maintenance media can help you minimize variation in your research. Each TeSR™ medium is based on published formulations[2-5] from the laboratory of Dr. James Thomson and offers unique features to fit your research needs.

Which TeSR™ Medium is Right for You?

mTeSR™1: Most-Published. cGMP Grade.

Proven

- Over 1500 peer-reviewed publications.
- Published protocols for a wide variety of hPSC applications including gene editing and cell scale-up in bioreactors.
- Published protocols for lineage-specific differentiation of hPSCs that have been maintained in mTeSR™1.

Robust

- Used to maintain thousands of human ES and iPS cell lines in over 60 countries for 10 years.
- Contains pre-screened BSA to stabilize medium, aid in lipid/nutrient transport, and protect cultures from cellular toxins and stresses.[3]

TeSR™2: Xeno-Free

Defined

- Modified formulation is similar to mTeSR™1, but manufactured with xeno-free components.[4]
- Compatible with published mTeSR™1 protocols for a wide variety of applications.
- Contains recombinant human albumin to aid in lipid/nutrient transport and protect cultures from cellular toxins and stresses.

TeSR™-E8™: Simplified and Animal Component-Free

Minimal Formulation

- Contains only the 8 most critical components required for hPSC maintenance.[2,5]
- Cutting edge, animal component-free formulation.
- 433X less protein than mTeSR™1.

mTeSR™3D: Suspension Culture

Scale-Up

- Optimized formulation for hPSC scale-up in suspension culture.
- Fed-batch culture system for a simplified workflow.
- Scale up to 10^9 high-quality, undifferentiated hPSCs in as few as 2 - 3 weeks.

mTeSR™1

Most-Published Feeder-Free hPSC Maintenance Medium

The same mTeSR™1 that you know and trust is now available as **cGMP**.

mTeSR™1 is a highly specialized and defined, serum-free and complete cell culture medium, with established protocols for applications ranging from gene editing and bioreactor expansion to lineage-specific differentiation. Proven to provide more consistent cultures with homogeneous, undifferentiated phenotypes, mTeSR™1 has been used to successfully maintain thousands of hPSC lines.

Known as the most reliable and consistent medium for hPSC culture, mTeSR™1 represents the highest quality, most versatile culture medium for hPSC-based models. It is uniquely compatible with a peerless array of specialized reagents developed to support all traditional and cutting-edge hPSC applications, providing both flexibility and seamlessly integrated workflows. 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 IND application.

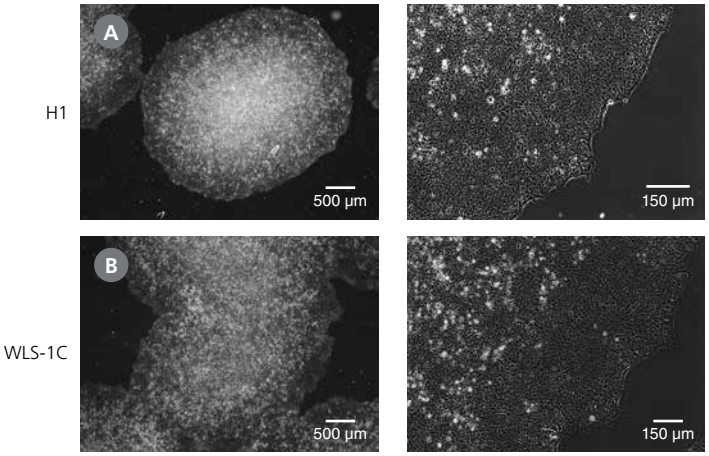


Figure 6. Normal Human ES and iPS Cell Morphology is Observed in mTeSR™1 Cultures

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.

Why Use mTeSR™1?

PROVEN. The most highly validated defined hPSC medium, backed by a decade of data. mTeSR™1 has been used in over 1500 peer-reviewed publications and is the medium of choice for feeder-free hPSC genome editing using CRISPR-Cas9.

FLEXIBLE. Supports multiple feeding and passaging timelines to suit your own schedule. mTeSR™1 can be combined with your cell culture matrix and passaging reagent of choice.

ROBUST. Contains pre-screened and quality-controlled components.

VERSATILE. Compatible with a peerless array of specialized reagents designed to support all traditional and cutting-edge applications.

cGMP. Ensures the highest quality and consistency for reproducible results.

mTeSR™1 is manufactured under cGMP guidelines (21 CFR 820).

PRODUCT	SIZE	CATALOG #
mTeSR™1	500 mL	85850
	1 L Kit	85857
	10 Kits	85870
	25 Kits	85875

TeSR™-E8™

Feeder-Free, Animal Component-Free Maintenance Medium

TeSR™-E8™ is a feeder-free, animal component-free (ACF) maintenance medium for hPSCs. TeSR™-E8™ is based on the E8 formulation[2,5] 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 Corning® Matrigel® hESC-qualified matrix, or with Vitronectin XF™ for a completely xeno-free system.

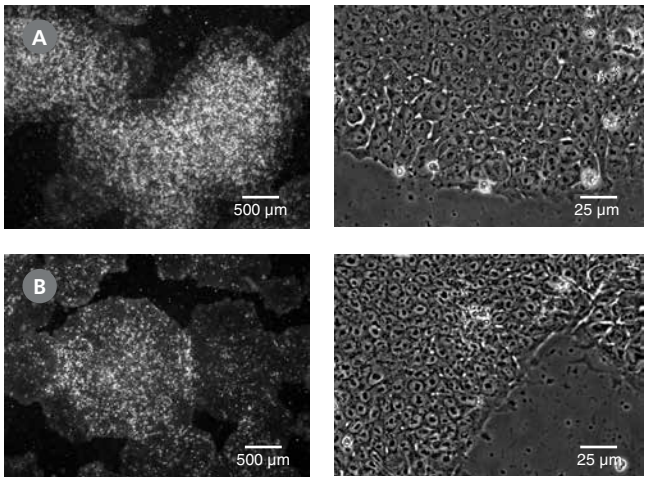


Figure 7. Normal Human ES and iPS Cell Morphology is Observed in TeSR™-E8™ Cultures

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.

Why Use TeSR™-E8™?

ANIMAL COMPONENT-FREE. Defined, cutting-edge formulation.

LOW PROTEIN. Minimal formulation contains only the most essential components required for hPSC maintenance.

PRODUCT	SIZE	CATALOG #
TeSR™-E8™	1 Kit	05990

Weekend-Free Schedule

mTeSR™1 and TeSR™-E8™ media have been validated for a flexible feeding schedule, allowing weekends free without compromising the quality of your hPSC cultures. See below for our suggested schedule, n which cells are only passaged once a week on Fridays, with no medium replacement required on either Saturday or Sunday. This passaging protocol has been rigorously tested and is suitable for routine use in long-term maintenance.

For more information and to see the data, refer to www.stemcell.com/weekendfreehPSC for the Technical Bulletin: Weekend-Free Culture of Human Pluripotent Stem Cells in mTeSR™1 or TeSR™-E8™ (Document #28071) or contact us to request a copy.

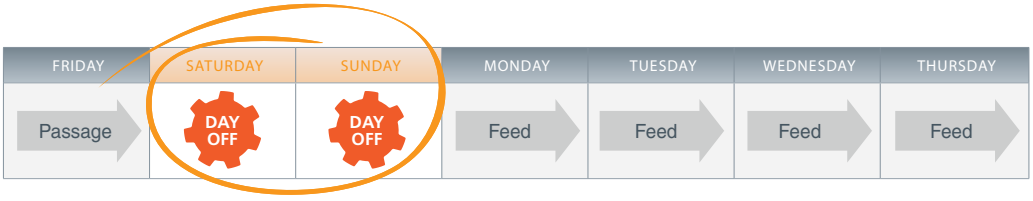


Figure 8. Overview of Weekend-Free Protocol

Naïve Induction and Maintenance

RSeT™ Medium

Defined Medium for Naïve-Like hPSCs

RSeT™ is a feeder-dependent, defined medium that reverts primed human pluripotent stem cells (hPSCs) and maintains cells in a naïve-like state (Figures 9,10). Developed under license from the Weizmann Institute of Science[6], this improved medium does not contain bFGF or TGFβ. With pre-screened quality components that ensure batch-to-batch consistency, RSeT™ produces robust cultures with phenotypes characteristic of naïve-like stem cells and markers associated with undifferentiated cells (Figure 9).

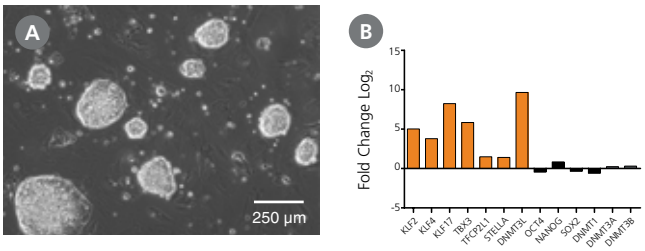


Figure 9. hPSCs Maintained in RSeT™ are Reverted to a Naïve-Like State and Express High Levels of Genes Associated with Naïve-Like hPSCs

(A) A representative image of hPSCs that reverted to a naïve-like state after being cultured in RSeT™ for 10 passages. During reversion, colonies change from a flat morphology to a domed morphology characteristic of naïve state hPSCs. (B) Expression of markers associated with naïve-like hPSCs (KLF2, KLF4, KLF17, TBX3, TCFP2L1, STELLA, and DNMT3L) in hPSCs that were reverted to a naïve-like state by culturing in RSeT™. Expression levels were measured by quantitative PCR (qPCR) and normalized to levels in primed hPSCs.

Why Use RSeT™ Medium?

EASY-TO-USE. Passage as single cells while maintaining normal karyotype.

NAÏVE-LIKE. Maintains pluripotency without inclusion of bFGF or TGFβ.

CONSISTENT. Defined medium contains pre-screened quality components.

TRANSGENE-FREE. No exogenous genes required for reversion to naïve-like state.

PRODUCT	SIZE	CATALOG #
RSeT™ Medium Kit	500 mL Kit	05970

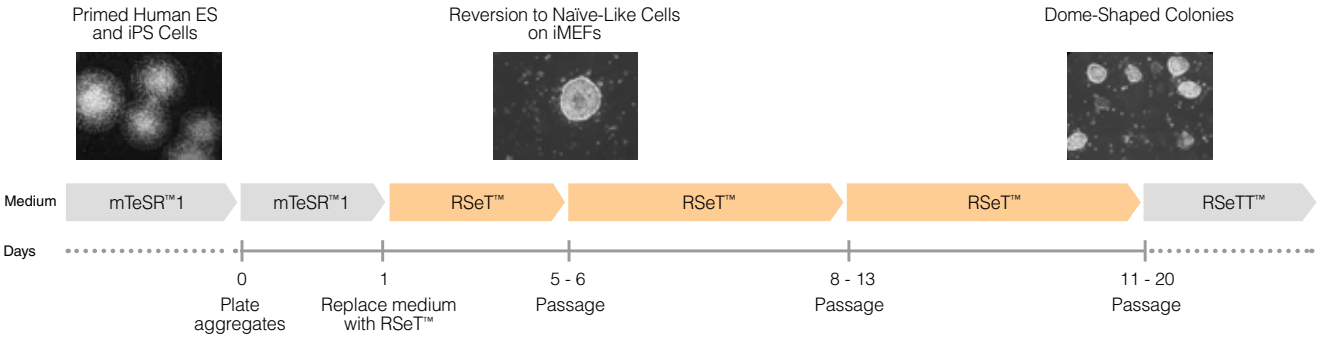


Figure 10. Schematic of Reversion of Primed to Naïve-Like hPSCs with RSeT™

Primed hPSCs are plated as aggregates in mTeSR™1 onto iMEFs. On day 1, mTeSR™1 is replaced with RSeT™, and the medium is exchanged daily. By day 5 or 6, the colonies are generally large enough to be passaged. During the initial culture in RSeT™ Medium, colonies expand and begin to adopt a domed shape characteristic of naïve-like stem cells and can continue to be propagated in RSeT™.

RSeT™ Feeder-Free Medium

Defined Medium for Feeder-Free Naïve-Like hPSCs

RSeT™ Feeder-Free is a defined medium that reverts primed hPSCs and maintains cells in a naïve-like state without the need of bFGF or feeder cells. Developed under license from the Weizmann Institute of Science6, this medium contains pre-screened quality components that ensure batch-to-batch consistency. RSeT™ Feeder-Free produces robust cultures with features of a naïve-like state such as tightly-packed, domed colonies with refractive edges (Figure 11A), and increased expression of key naïve-associated transcripts (Figure 11B). This newly improved formulation enables efficient reversion to a naïve-like state as early as passage 1, without the inherent variability and burden associated with the use of feeder cells.

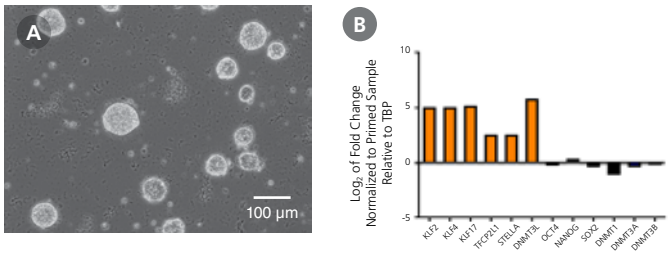


Figure 11. hPSCs Maintained in RSeT™ Feeder-Free are Reverted to a Naïve-Like State and Express High Levels of Naïve-Associated Genes

(A) A representative image of hPSCs that reverted to a naïve-like state after being cultured in RSeT™ Feeder-Free 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. Expression levels were measured by qPCR and normalized to levels in primed hPSCs.

Why Use RSeT™ Feeder-Free Medium?

CONSISTENT. Feeder-free, defined formulation maintains naïve-like pluripotency without inclusion of bFGF.

TRANSGENE-FREE. No exogenous genes required for reversion to naïve-like state.

EFFICIENT. Reversion to naïve-like state as early as passage 1.

EASY-TO-USE. Simplified formulation and straightforward reversion protocol.

PRODUCT	SIZE	CATALOG #
RSeT™ Feeder-Free Medium Kit	500 mL Kit	05975

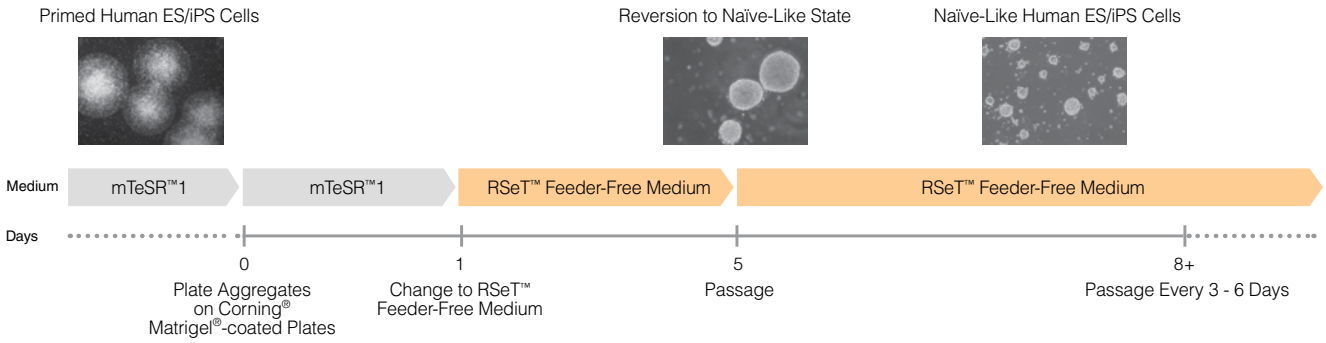


Figure 12. Schematic of Reversion of Primed to Naïve-Like hPSCs with RSeT™ Feeder-Free

Primed hPSCs are plated as aggregates in mTeSR™1. On day 1, mTeSR™1 is replaced with RSeT™ Feeder-Free, 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, 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.

NaïveCult™

Defined Medium for Transgene-Free Induction and Expansion of Naïve Reset hPSCs

NaïveCult™ is a defined media system that generates transgene-free reset naïve hPSCs from primed hPSCs and allows for their continual maintenance (Figure 15). This product line was developed under license from Cambridge Enterprises.[7] NaïveCult™ contains pre-screened quality components to work consistently across multiple human ES and iPS cell lines.

Why Use NaïveCult™?

NAÏVE. Maintains hPSCs with high expression of naïve-associated genes.

ROBUST. Works consistently across multiple human ES and iPS cell lines.

TRANSGENE-FREE. No exogenous genes required for reversion to the naïve state.

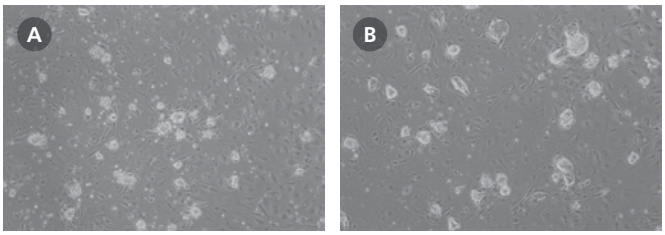


Figure 13. Human ES and iPS Cells Can Be Reverted to a Naïve State

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.[7-9]

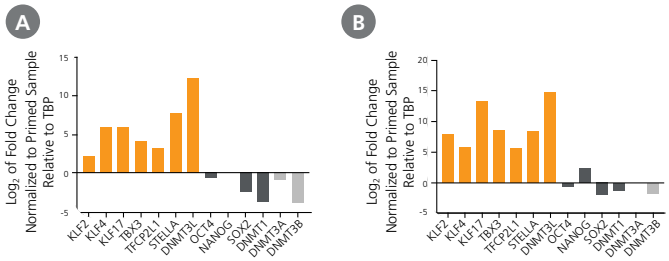


Figure 14. hPSCs Cultured in the NaïveCult™ Media System Express High Levels of Factors Associated with Naïve hPSCs[7-9]

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.

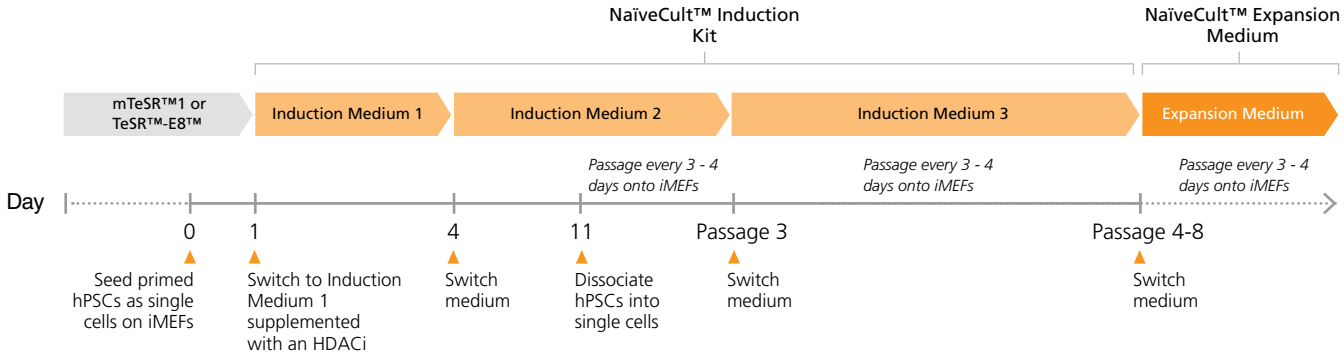


Figure 15. Schematic of Reversion of Primed to Naïve-Like hPSCs in NaïveCult™

Primed hPSCs are plated as single cells on iMEFs and treated with Rho-kinase inhibition (10 µM Y-27632) for 24 hours in hypoxic conditions. On day 1, medium is changed to Induction Medium 1 supplemented with a histone deacetylase inhibitor (HDACi) and cells are cultured for 3 days. On day 4, medium is changed to Induction Medium 2 and cells are cultured until day 11. On day 11, hPSCs are passaged as single cells onto fresh iMEFs and subsequently passaged twice in Induction Medium 2. From passage 3, hPSCs are cultured in Induction Medium 3. 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.

hPSC Naïve State qPCR Array

The hPSC Naïve State qPCR Array 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).

PRODUCT	SIZE	CATALOG #
NaïveCult™ Induction Kit	1 Kit	05580
NaïveCult™ Expansion Medium	1 Kit	05590
hPSC Naïve State qPCR Array	96-well Plate	07521

Scale-Up

mTeSR™3D

hPSC Suspension Culture Medium

mTeSR™3D is the newest member of the TeSR™ family of media and has been specifically developed for expansion and scale-up of human pluripotent stem cells (hPSCs) as aggregates in suspension culture. It is optimized as a fed-batch culture system, in which required nutrients are added daily, eliminating the need for daily medium exchanges.

hPSCs expanded in the mTeSR™3D fed-batch culture system have robust growth and maintain high expression of pluripotent stem cell markers (Fig 17). hPSCs cultured in mTeSR™3D retain trilineage differentiation ability and show robust differentiation to all three germ lineages.

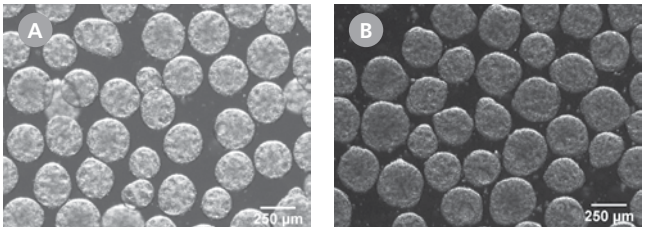


Figure 16. Morphology of hPSC Aggregates Cultured in mTeSR™3D

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. Scale bars indicate size.

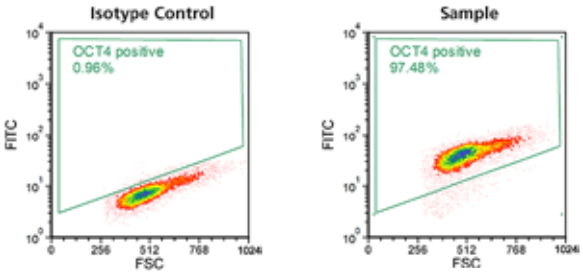


Figure 17. OCT4 Expression of hPSCs Cultured in mTeSR™3D

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

Why Use mTeSR™3D?

OPTIMIZED. Part of the mTeSR™ family, mTeSR™3D is optimized for hPSC suspension culture.

SIMPLIFIED WORKFLOW. Fed-batch strategy provides a simplified culture system.

DEFINED. Serum-free culture system with no microcarriers or external matrix required.

SCALE-UP. Easily produce up to 1 x 109 hPSCs in as little as 2 - 3 weeks.

VERSATILE. Compatible with a variety of suspension culture vessels.

COST EFFECTIVE. Significant cost savings in both media and labor.

PRODUCT	SIZE	CATALOG #
mTeSR™3D*	1 Kit	03950

*Includes the mTeSR™3D Seed Medium (basal and 5X supplement) and mTeSR™3D Feed Medium (Feed Supplements A and B). Components not available for individual sale.

Global Exclusive Licensing

mTeSR™3D is manufactured and sold under global exclusive license from Accellta for culture medium for hPSCs in suspension under feeder-free, non-adherent conditions.

Matrices

Vitronectin XF™ and CellAdhere™ Laminin-521 are defined, xeno-free cell culture matrices that support the growth and differentiation of human pluripotent stem cells (hPSCs). When used with mTeSR™1, TeSR™-E8™ or TeSR™2, they provide a defined culture system for cell maintenance under feeder-free conditions. These systems allow complete control over the culture environment, resulting in more consistent cell populations and reproducible results in downstream applications.

Vitronectin XF™

For Growth and Differentiation of hPSCs Under Serum-Free, Feeder-Free Conditions

Developed and manufactured by Primorigen Biosciences, Inc., Vitronectin XF™ is an effective alternative to Corning® Matrigel®. Human ES and iPS cells cultured on Vitronectin XF™ retain pluripotency and normal colony morphology, without the need for an adaptation step (Figure 18).

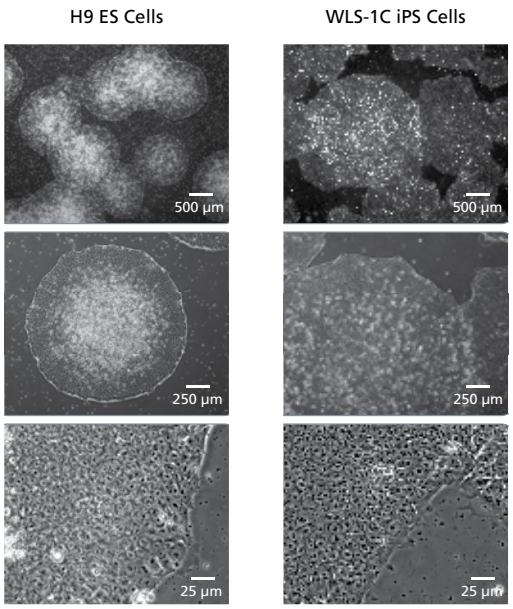


Figure 18. Morphology of Human ES and iPS Cells Cultured on Vitronectin XF™ Cell Culture Matrix in TeSR™-E8™

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 Corning® Matrigel® 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 Corning® Matrigel®, which are more diffuse and irregularly shaped.

Why Use Vitronectin XF™ and CellAdhere™ Laminin-521?

DEFINED. Recombinant human protein.

EASY-TO-USE. Simple, room temperature handling without the inconvenience of matrix gelling.

COMPATIBLE. Use with any TeSR™ family medium to maintain hPSCs.

XENO-FREE. Create a completely xeno-free system when used with TeSR™2 or TeSR™-E8™.

CellAdhere™ Laminin-521

For Long-Term Maintenance in Feeder-Free Conditions

Using CellAdhere™ Laminin-521 as a cell culture matrix increases single-cell attachment and survival compared to other matrices and does not require the addition of apoptotic inhibitors during plating.

PRODUCT	SIZE	CATALOG #
Vitronectin XF™ with GCDR	1 Kit	07190
Vitronectin XF™ with ReLeSR™	1 Kit	07191
Vitronectin XF™	2 mL	07180
CellAdhere™ Laminin-521	100 µg	77003
	10 x 100 µg	77004

*Kit contains Vitronectin XF™, CellAdhere™ Dilution Buffer, Gentle Cell Dissociation Reagent and Non-Tissue Culture-Treated 6-Well Plates.

Dissociation Reagents

ReLeSR™ and Gentle Cell Dissociation Reagent

For Enzyme-Free hPSC Passaging

ReLeSR™ selectively detaches undifferentiated cells from human pluripotent stem cell (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. 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) is an enzyme-free reagent suitable for the dissociation of hPSCs into cell aggregates for routine passaging or into a single-cell suspension.

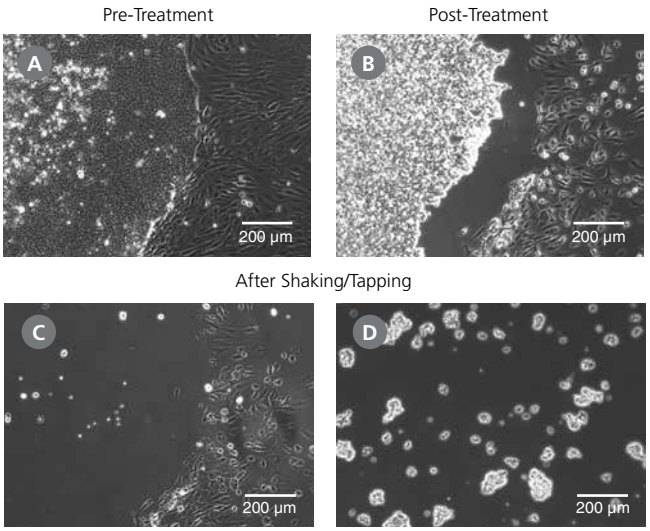


Figure 19. ReLeSR™ Selectively Detaches Undifferentiated Cells from hPSC Cultures Without Manual Selection and Generates Optimally Sized Aggregates

(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.

Why Use ReLeSR™ and GCDR?

SCALABLE. Compatible with culture processes involving closed vessels.

CHEMICALLY DEFINED. Enzyme-free formulation minimizes variability.

EASY-TO-USE. Easily generates optimally sized aggregates without manual scraping.

COMPATIBLE. Use with any TeSR™ family media, Vitronectin XF™ and Corning® Matrigel®.

ACCUTASE™

For Creation of Single-Cell Suspensions

ACCUTASE™ is a cell detachment solution of proteolytic and collagenolytic enzymes, and is useful for the routine detachment of cells from standard tissue culture plasticware and adhesion-coated plasticware. ACCUTASE™ does not contain mammalian or bacterial-derived products.

Dispase

For Enzymatic Dissociation

Dispase is a commonly used enzyme preparation recommended for passaging hPSCs maintained in feeder-free conditions on Corning® Matrigel®.

PRODUCT	SIZE	CATALOG #
ReLeSR™	100 mL	05872
Gentle Cell Dissociation Reagent	100 mL	07174
ACCUTASE™	100 mL	07923
Dispase	1 U/mL	07923

Genome Editing

CloneR™

Enhancing the Cloning Efficiency and Single-Cell Survival of hPSCs

Genome editing of human pluripotent stem cells (hPSCs) relies heavily on the survival of single cells to establish clonal lines. CloneR™ is a defined, serum-free supplement formulated for enhancing the cloning efficiency and single-cell survival of hPSCs, especially under clonal and low-density seeding conditions (Figure 20). Designed for use in feeder-free culture systems, this flexible supplement is compatible with TeSR™ maintenance medium, a range of cell culture matrices, and cell lines (Figure 21). Unlike current methods, CloneR™ enables the robust generation of clonal hPSC lines without single-cell adaptation, thus minimizing the risk of acquiring genetic abnormalities.

PRODUCT	SIZE	CATALOG #
CloneR™	10 mL	05888
	5 x 10 mL	05889

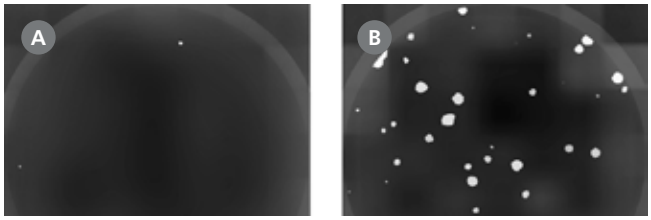
Why Use CloneR™?

EFFICIENT. Increased single-cell survival at low and clonal densities.

EASY-TO-USE. No adaptation to single-cell passaging required.

FLEXIBLE. Compatible with any TeSR™ maintenance medium and your choice of cell culture matrix.

ROBUST. Increased cloning efficiency across multiple human ES and iPS cell lines.



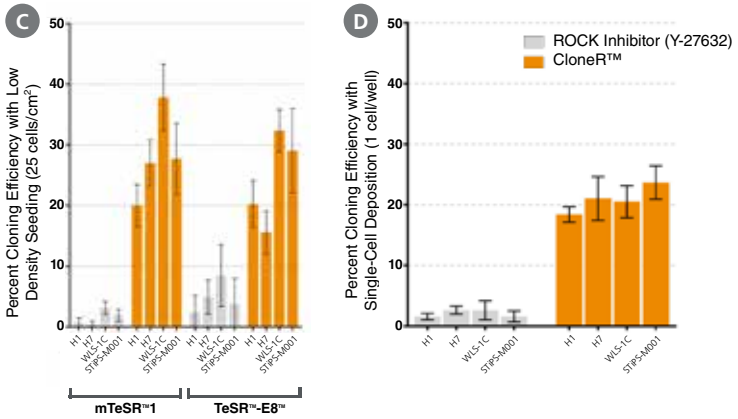


Figure 20. CloneR™ Increases the Cloning Efficiency of hPSCs at Low Seeding Densities

(A-D) hPSCs plated in TeSR™ medium supplemented with CloneR™ demonstrated significantly increased cloning efficiencies compared to cells plated in TeSR™ containing ROCK inhibitor (10 µM Y-27632). (A,B) Representative images of alkaline phosphatase-stained colonies at day 7 in individual wells of a 12-well plate. Human H1 ES cells were seeded at clonal density (100 cells/well, 25 cells/cm^2) in mTeSR™1 supplemented with (A) ROCK inhibitor or (B) CloneR™ on Vitronectin XF™ cell culture matrix. Cells were seeded (C) at clonal density (25 cells/cm^2) in mTeSR™1 or TeSR™-E8™ and (D) by single-cell deposition using FACS (seeded at 1 cell/well) in mTeSR™1.

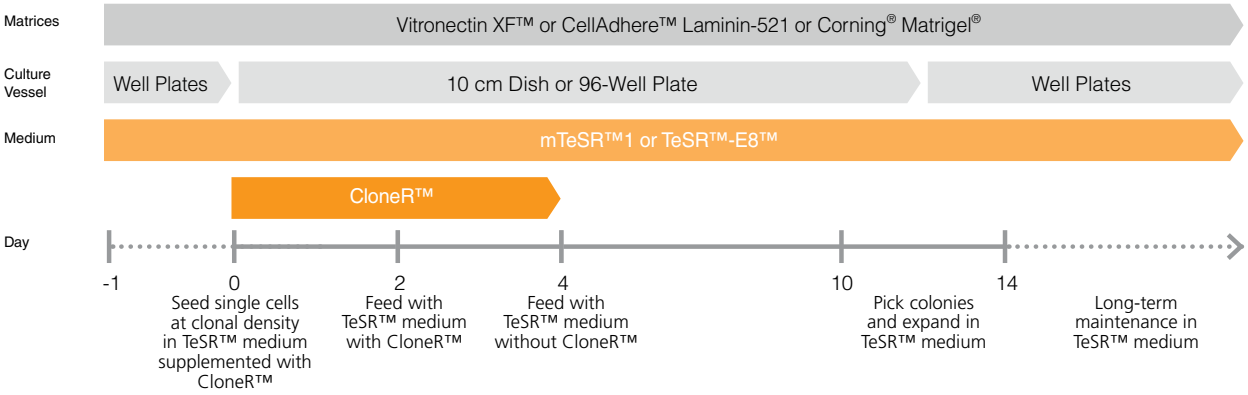


Figure 21. hPSC Single-Cell Cloning Workflow with CloneR™

On day 0, hPSCs are seeded as single cells at clonal density (e.g. 25 cells/cm^2) or sorted at 1 cell per well in 96-well plates in TeSR™ (mTeSR™1 or TeSR™-E8™) medium supplemented with CloneR™. On day 2, the cells are fed with TeSR™ medium containing CloneR™ supplement. From day 4, cells are maintained in TeSR™ medium without CloneR™. Colonies are ready to be picked between days 10 - 14. Clonal cell lines can be maintained long-term in TeSR™ medium.

ArciTect™

For Genome Editing of hPSCs Using the CRISPR-Cas9 System

The ease-of-use and versatility of CRISPR-Cas9 has revolutionized hPSC research. This technological advance has enabled gene knockout and introduction or correction of specific mutations to further understanding of how individual genes and/or genetic variants impact biology and disease pathogenesis. ArciTect™ is designed to fully support genome editing in hPSCs, providing you with a rapid, flexible, and precise system to modify the genome as you see fit. From cell culture and single-cell survival to experimental design, detection, and validation of editing efficiency, our continuously expanding toolkit contains qualified solutions for every step in the hPSC genome editing workflow. Our optimized and validated protocol (Document #27084) is specifically designed to work seamlessly with ArciTect™ offerings to minimize troubleshooting and maximize experimental success.

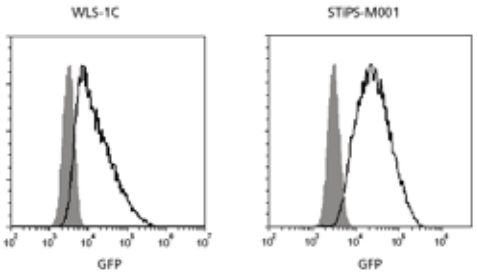


Figure 22. ArciTect™ Cas9-eGFP Detection by Flow Cytometry

WLS-1C (left) or STiPS-M001 (right) iPS cells were transfected with RNP complex containing ArciTect™ Cas9-eGFP; eGFP was detected by flow cytometry 24 hours after transfection. Filled histogram: Non-transfected control; Solid line histogram: Cas9-eGFP-transfected cells.

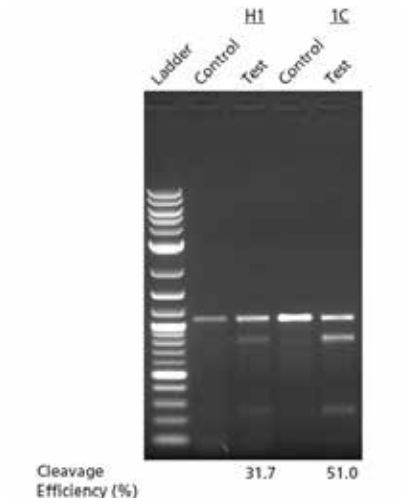


Figure 23. INDEL Detection by the ArciTect™ T7 Endonuclease I Assay

H1 ES cells or WLS-1C iPS cells were edited using the ArciTect™ Human HPRT Positive Control Kit and INDEL formation (percent [%] cleavage efficiency) was assessed using the ArciTect™ T7 Endonuclease I Kit. Control: Non-transfected cells; Test: HPRT-edited.

Why Use ArciTect™?

CUSTOMIZABLE. Design crRNA to target your sequence of interest.

FLEXIBLE. Multiple variations of Cas9 to suit your specific genome editing needs.

RAPID. No need for transcription and translation.

REDUCED OFF-TARGET EFFECTS. Timely degradation of the RNP complex to minimize potential off-target cutting.

PRODUCT	SIZE	CATALOG #
ArciTect™ Cas9 Nuclease	50 µg	76001
	100 µg	76002
	300 µg	76004
ArciTect™ Cas9-eGFP Nuclease	50 µg	76005
	100 µg	76006
ArciTect™ Cas9 Nickase	10 µg	76007
	50 µg	76008
	100 µg	76009
ArciTect™ crRNA	2 nmol	76010
	10 nmol	76011
	20 nmol	76012
ArciTect™ tracrRNA Kit	5 nmol Kit	76016
	10 nmol Kit	76017
	20 nmol Kit	76018
ArciTect™ Annealing Buffer	1 mL	76020
ArciTect™ Human HPRT Positive Control Kit	1 Kit	76013
ArciTect™ T7 Endonuclease I Kit	25 Reactions	76021
	125 Reactions	76022

Cell Quality Characterization

hPSC Genetic Analysis Kit

qPCR Analysis Kit to Detect the Majority of Karyotypic Abnormalities Reported in Human ES and iPS Cells

The hPSC Genetic Analysis Kit contains primer/probe mixes to detect the majority of karyotypic abnormalities reported in human ES and 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.

PRODUCT	SIZE	CATALOG #
hPSC Genetic Analysis Kit	1 Kit	07550

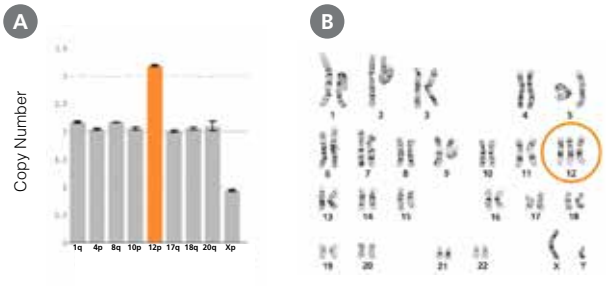


Figure 24. The hPSC Genetic Analysis Kit Identifies Chromosome 12 Trisomy

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

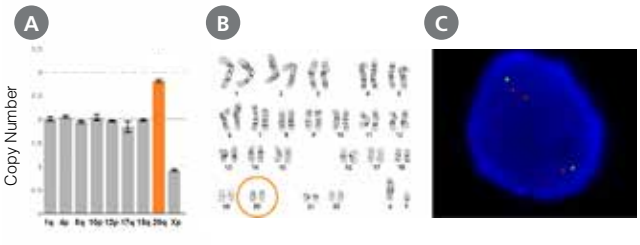


Figure 25. The hPSC Genetic Analysis Kit Identifies Chromosome 20q11.21 Duplication

Chromosome 20q duplication in 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).

Why Use the hPSC Genetic Analysis Kit?

TARGETED. Designed to detect the majority of karyotypic abnormalities observed in human pluripotent stem cell (hPSC) cultures.

RAPID. From cells in culture to results within one day.

COST-EFFECTIVE. Low cost per sample enables more frequent screening of multiple samples.

CONVENIENT. Online hPSC Genetic Analysis Tool for streamlined data analysis and interpretation.

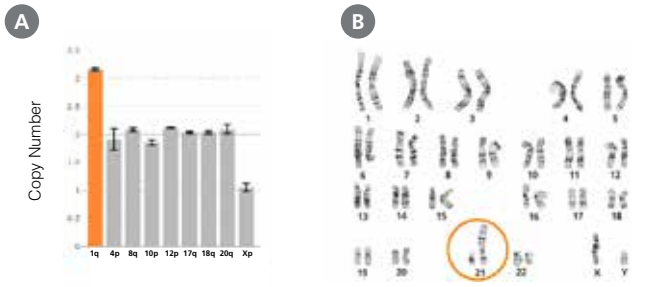


Figure 26. The hPSC Genetic Analysis Kit Identifies Chromosome 1 Duplication via Unbalanced Translocation

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.

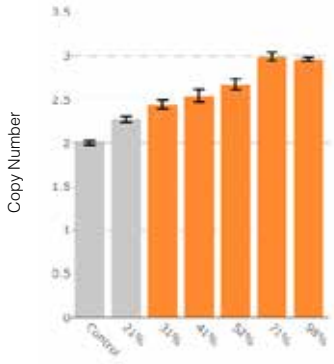


Figure 27. The hPSC Genetic Analysis Kit Identifies Abnormalities in Cultures with Approximately 30% Mosaicism

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

Directed Differentiation for Validation of Pluripotency

The STEMdiff™ Trilineage Differentiation Kit 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: ectoderm, mesoderm and endoderm. 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.

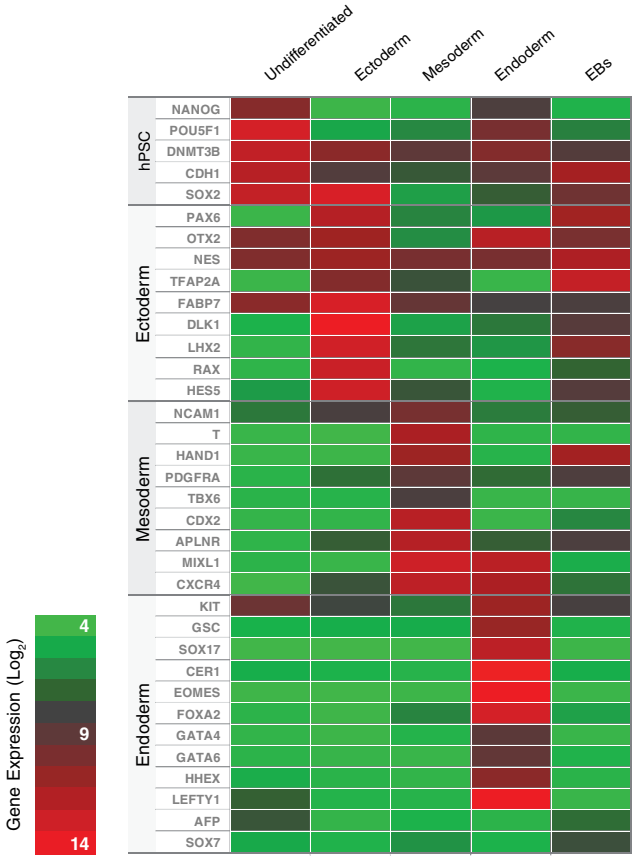


Figure 28. Molecular Analysis of Cultures Differentiated with the STEMdiff™ Trilineage Differentiation Kit Show Strong Separation of Lineage-Specific Markers

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.

Why Use the STEMdiff™ Trilineage Differentiation Kit?

ROBUST. Reproducible directed differentiation to all three germ layers across multiple pluripotent cell lines.

CLEAR. Easy-to-interpret assay results.

DEFINED. Complete, defined cell culture media.

EFFICIENT. Standardized, one-week protocol.

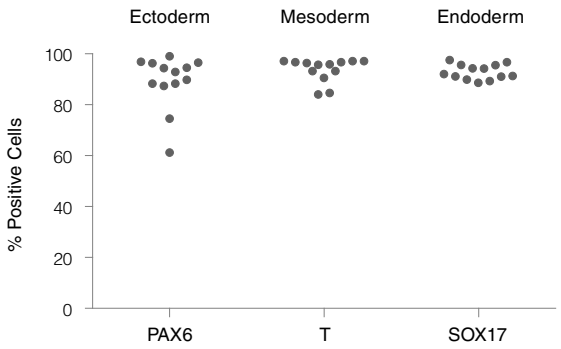


Figure 29. The STEMdiff™ Trilineage Differentiation Kit Promotes Efficient Differentiation to All Three Germ Layers

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 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).

PRODUCT	SIZE	CATALOG #
STEMdiff™ Trilineage Differentiation Kit	1 Kit	05230
hPSC Trilineage Differentiation qPCR Array	384-well Plate	07515

Cryopreservation

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.[10-13] mFreSR™ serum-free medium is optimized for cryopreservation of hPSCs as aggregates. FreSR™-S 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 (Figures 30,31).

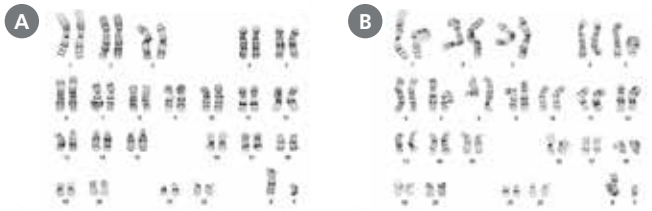


Figure 30. hPSCs Frozen and Thawed as Single Cells with FreSR™-S Display a Normal Karyotype

Karyograms of WLS-4D1 iPS cells that were frozen and thawed as single cells using FreSR™-S. (A) Thawed cells were seeded into culture containing TeSR™-E8™ medium and 10 µM Y-27632 and maintained as aggregates for five passages. (B) hPSCs were also subjected to a second freeze-thaw cycle as single cells with FreSR™-S and cultured for five passages as aggregates prior to collection of karyotype data.

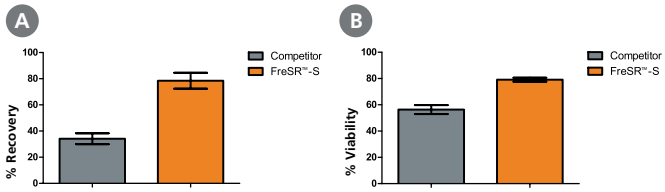


Figure 31. High Viability and Recovery of Cells Stored in FreSR™-S

hPSCs cryopreserved as single cells using FreSR™-S have (A) higher post-thaw recovery (number of cells recovered per number of cells frozen) and (B) maintain higher viability (number of live cells per total number of cells) compared with competitor medium. All data values are plotted as percentages (n = 18, $p < 0.0001$ for each).

Why Use FreSR™ Media?

COMPATIBLE. Optimized for cells cultured in TeSR™ family media.

HIGH VIABILITY. Cells have higher post-thaw recovery and maintain high viability.

ROBUST. hPSCs retain undifferentiated cell markers and expansion capability.

CryoStor® CS10

Storage and cryopreservation of cells and tissues are important parts of the workflow for biological research. CryoStor® CS10 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.

Why Use CryoStor® CS10?

ANIMAL COMPONENT-FREE. Chemically defined formulation.

REGULATORY COMPLIANCE. cGMP-manufactured with USP grade components; US FDA Drug Master File.

CONVENIENT. Ready-to-use and available in several size options.

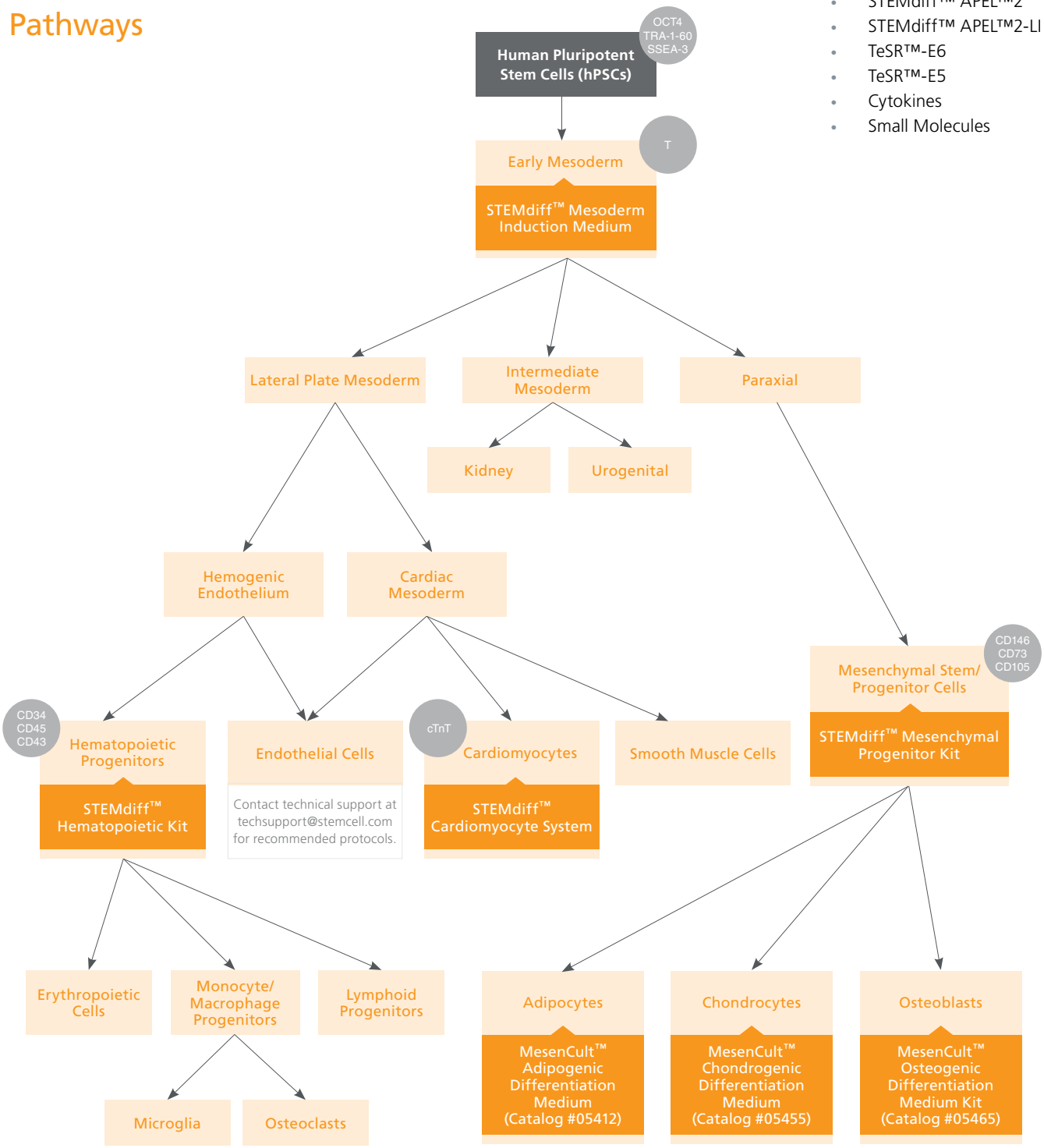
PRODUCT	SIZE	CATALOG #
mFreSR™	50 mL	05855
	10 x 5 mL Tubes	05854
FreSR™-S	50 mL	05859
CryoStor® CS10	100 mL	07930
	5 x 16 mL Vials	07931
	1000 mL Bag	07940
	100 mL Bag	07955
	5 x 10 mL Vials	07959
	16 x 10 mL Vials	07952

Differentiation

Mesoderm Differentiation Pathways

Flexible Products for Differentiation

- STEMdiff™ APEL™2
- STEMdiff™ APEL™2-LI
- TeSR™-E6
- TeSR™-E5
- Cytokines
- Small Molecules



STEMdiff™ Mesoderm Induction Medium

Xeno-Free Differentiation to Early Mesoderm

STEMdiff™ Mesoderm Induction Medium (MIM) is a defined, xeno-free medium for generation of early mesoderm cells from human ES and iPS cells. Protocols for mesodermal differentiation can be difficult and inconsistent, therefore, use the short and simple STEMdiff™ MIM monolayer protocol to differentiate your hPSCs.

STEMdiff™ MIM produces a cell population enriched for early mesoderm, as indicated by positive expression of Brachyury (T), MIXL1 and NCAM markers (Figure 32).

Why Use STEMdiff™ MIM?

XENO-FREE. Defined formulation for mesoderm induction.

RAPID. Induction of mesoderm after only 2 - 4 days of differentiation.

EFFICIENT. Reproducible differentiation of multiple human ES and iPS cell lines.

MULTIPOTENT. Generates early mesoderm cells that are capable of differentiation to multiple downstream cell types.

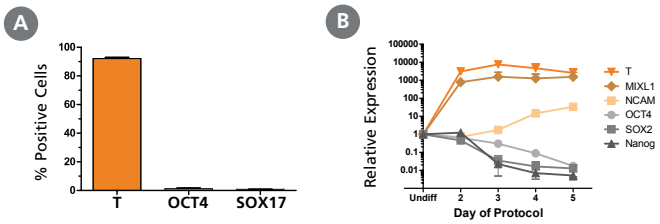


Figure 32. STEMdiff™ MIM Efficiently Generates a Homogenous Population of Early Mesoderm Cells

(A) Data showing marker expression characteristic of early mesoderm (positive Brachyury (T) expression and negative OCT4 and SOX 17 expression) on day 5 of the protocol. Data expressed as a mean percentage of cells expressing each marker ± SD, n = 33 (T, OCT4); n = 5 (SOX17). (B) Expression of undifferentiated cell markers (OCT4, SOX2, NANOG) and early mesoderm markers (T, MIXL1, NCAM), measured by qPCR and normalized to levels in undifferentiated cells; n = 2.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Mesoderm Induction Medium	100 mL	05220
	500 mL	05221

STEMdiff™ Mesenchymal Progenitor Kit

Mesenchymal Differentiation

The STEMdiff™ Mesenchymal Progenitor Kit is optimized for the efficient and reproducible derivation of mesenchymal progenitor cells (MPCs) from human ES or iPS cells. This kit contains animal component-free (ACF) induction medium, expansion medium and attachment substrate for the derivation and expansion of MPCs. It uses a simple monolayer protocol to generate MPCs under feeder-free conditions in three weeks. Human ES- or iPS cell-derived MPCs are capable of long-term expansion (Figure 33). The derived MPCs are characterized by strong expression of cell-surface markers CD73, CD 90, CD105 and CD146, and lack expression of CD34, CD45 and CD144.

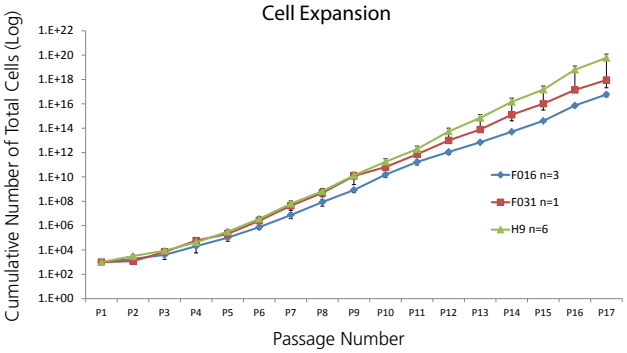


Figure 33. Cell Expansion of MPCs Derived from Human ES (H9) and iPS (STiPS-F016 and -F031) Cells in MesenCult™-ACF Medium

The average cell expansion per passage over 17 passages for MPCs derived from human ES and iPS cell lines were approximately between 9- to 10- fold.

Why Use the STEMdiff™ Mesenchymal Progenitor Kit?

DEFINED. Serum-free and ACF formulation.

ROBUST. Efficient and reproducible generation of MPCs from multiple human ES or iPS cell lines.

FAST. Rapid derivation of MPCs in three weeks.

FUNCTIONAL. Generates MPCs capable of long-term expansion and differentiation to adipocytes, osteoblasts and chondrocytes.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Mesenchymal Progenitor Kit	1 Kit	05240

STEMdiff™ Hematopoietic Kit

For the Generation of Hematopoietic Progenitor Cells

The STEMdiff™ Hematopoietic Kit consists of serum-free basal medium and supplements designed for the generation of hematopoietic progenitor cells (HPCs) from human ES and iPS cells. Optimized for a standardized, 12-day differentiation protocol (Figure 35), this kit supports robust differentiation of hPSCs into HPCs that can be identified by the expression of CD34 and CD45 (Figure 34) and by the ability to form hematopoietic colonies of multiple lineages in colony-forming unit (CFU) assays with MethoCult™ medium.

This kit is formulated for use in feeder-free conditions, optimized for the differentiation of hPSCs maintained in TeSR™ medium and compatible with multiple human ES and iPS cell lines. After differentiation the resulting HPCs may be used for downstream assays or quantified in a CFU assay with MethoCult™ SF H4636 (Catalog #04636) medium, designed specifically for use with hPSC-derived HPCs, or MethoCult™ H4435 Enriched (Catalog #04435) medium.

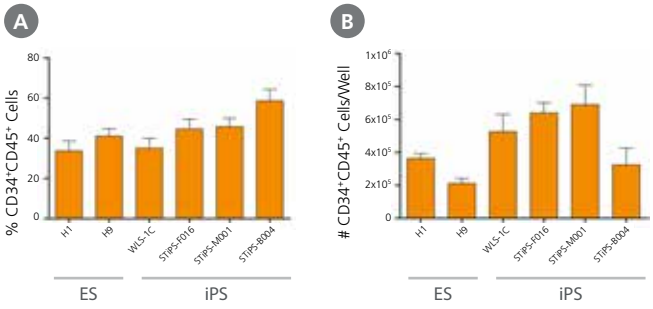


Figure 34. Efficient and Robust Generation of $CD34^+CD4^+$ HPCs

Human ES and iPS cells were cultured for 12 days in single wells of 12-well plates using the STEMdiff™ Hematopoietic Kit. At the end of the culture period, cells in suspension were harvested, stained and analyzed by flow cytometry for the expression of hematopoietic cell surface markers CD34 and CD45. (A,B) Percentages and total numbers of $CD34^+CD45^+$ cells in cultures of human ES or iPS cells are shown for 6 cell lines. Data shown as mean ± SEM; n ≥ 3.

Why Use the STEMdiff™ Hematopoietic Kit?

CONSISTENT. Serum-free and feeder-free formulation.

EASY-TO-USE. Simple monolayer protocol produces HPCs in suspension for easy harvest.

RAPID. Generation of HPCs in 12 days.

HIGH YIELD. One kit typically generates 4 - 18 million $CD34^+CD45^+$ HPCs.

FLEXIBLE. Robust generation of HPCs across multiple human ES and iPS cell lines.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Hematopoietic Kit*	1 Kit	05310

*Kit includes basal medium and supplements A and B.

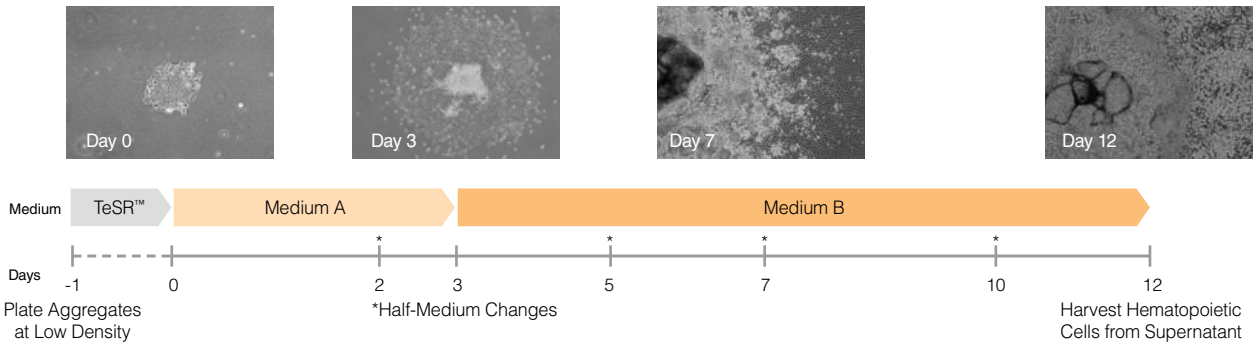


Figure 35. Hematopoietic Differentiation Protocol

One day before the differentiation protocol, hPSC colonies are harvested and seeded as small aggregates (100 - 200 µm in diameter) at 10 - 20 aggregates/cm^2 in mTeSR™1 or TeSR™-E8™. After one day, TeSR™ medium is replaced with Medium A to begin inducing the cells towards a mesoderm-like state (day 0). On day 2, a half-medium change is performed with fresh Medium A. On day 3, the medium is changed to Medium B with half-medium changes on days 5, 7 and 10 to promote further hematopoietic differentiation. Typically, by day 12, large numbers of HPCs can be harvested from the culture supernatant.

STEMdiff™ Cardiomyocyte System

Optimized for the Entire hPSC-Derived Cardiomyocyte Research Workflow

The STEMdiff™ Cardiomyocyte Differentiation Kit consists of defined, serum-free basal media and reagents designed for the generation of cardiomyocytes from hPSCs. Optimized for a standardized, 15-day differentiation protocol, this kit supports robust differentiation of hPSCs into cardiomyocytes that can be identified by the expression of a key marker cardiac troponin T (cTnT) (Figure 36). Contracting hPSC-derived cardiomyocytes can be seen as early as day 8. This kit is formulated for use in feeder-free conditions, optimized for the differentiation of hPSCs maintained in mTeSR™1 or TeSR™-E8™ and compatible with multiple hPSC lines. After differentiation, the resulting cardiomyocytes may be used for additional downstream assays, such as disease modeling, drug discovery, and cardiotoxicity screening.

Following hPSC differentiation to cardiomyocytes, the hPSC-derived cardiomyocytes can be maintained long-term in the STEMdiff™ Cardiomyocyte Maintenance Kit. The STEMdiff™ Cardiomyocyte Dissociation Kit allows for standardized harvesting of hPSC-derived cardiomyocytes that are ready for use in downstream applications, analysis and cryopreservation. hPSC-derived cardiomyocytes can be cryopreserved using the STEMdiff™ Cardiomyocyte Freezing Medium. The STEMdiff™ Cardiomyocyte Support Medium maintains the viability of cardiomyocytes after thawing or during dissociation, harvesting or re-plating.

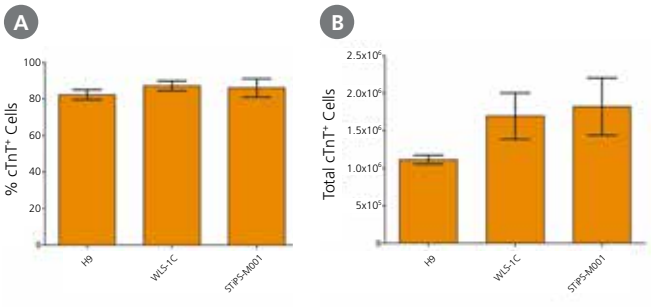


Figure 36. Efficient and Robust Generation of cTnT-Positive Cardiomyocytes

hPSCs were cultured for 15 days in single wells of 12-well plates using the STEMdiff™ Cardiomyocyte Differentiation Kit. At the end of the culture period, cells were harvested and analyzed by flow cytometry for expression of cell marker cTnT. (A) Percentages and (B) total numbers of cells expressing cTnT in cultures of human ES (H9) or iPS (WLS-1C and STiPS-M001) cells are shown. Data shown as mean ± SEM; n = 3.

Why Use the STEMdiff™ Cardiomyocyte Differentiation Kit?

COMPLETE. Supports the entire hPSC-derived cardiomyocyte workflow.

EASY-TO-USE. Simple monolayer protocol produces cardiomyocytes in 15 days.

HIGH YIELD. One kit generates over 50 million cardiomyocytes expressing cTnT.

STANDARDIZED. Robust performance with minimal variability across multiple hPSC lines.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Cardiomyocyte Differentiation Kit*	1 Kit	05010
STEMdiff™ Cardiomyocyte Maintenance Kit	1 Kit	05020
STEMdiff™ Cardiomyocyte Dissociation Kit	1 Kit	05025
STEMdiff™ Cardiomyocyte Support Medium	250 mL	05027
STEMdiff™ Cardiomyocyte Freezing Medium	50 mL	05030

*Kit includes STEMdiff™ Cardiomyocyte Differentiation Basal Medium and 10X Supplements A, B and C and STEMdiff™ Cardiomyocyte Maintenance Basal Medium and 50X Supplement.

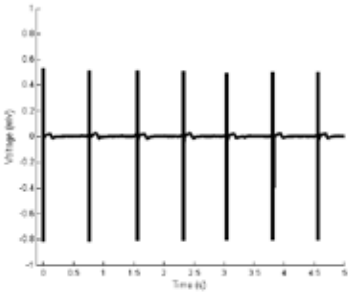
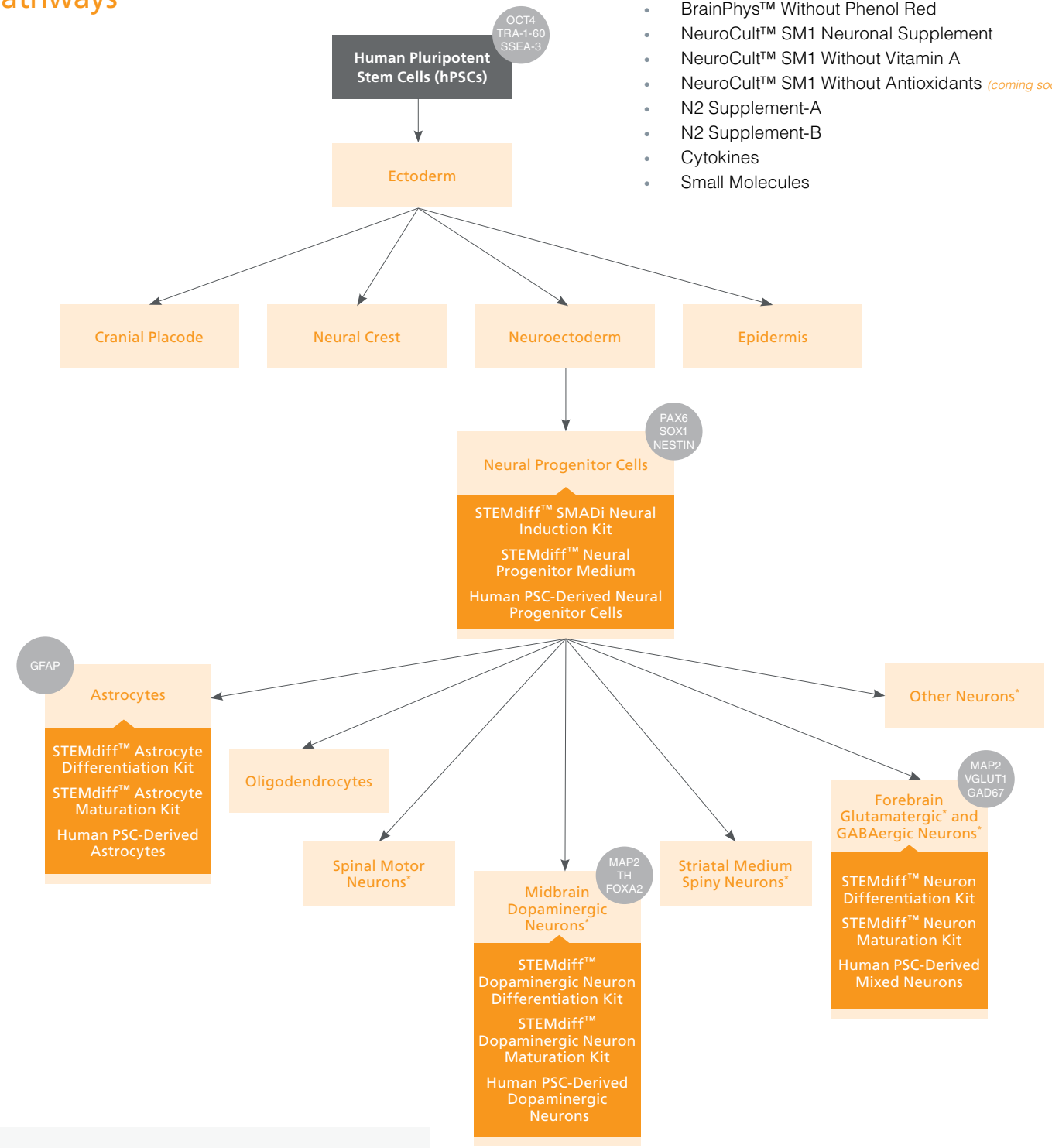


Figure 37. hPSC-Derived Cardiomyocytes Exhibit a Robust and Stable Excitability Profile

Microelectrode array (MEA) voltage recordings of cardiomyocytes (day 27) derived from hPSCs generated and maintained with the STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits. The hPSC-derived cardiomyocytes have a characteristic electrical profile and stable beat rate. A large depolarization spike followed by a smaller repolarization deflection are observed.

Ectoderm Differentiation Pathways



Flexible Products for Differentiation

- BrainPhys™ Neuronal Medium
- BrainPhys™ Without Phenol Red
- NeuroCult™ SM1 Neuronal Supplement
- NeuroCult™ SM1 Without Vitamin A
- NeuroCult™ SM1 Without Antioxidants *(coming soon)*
- N2 Supplement-A
- N2 Supplement-B
- Cytokines
- Small Molecules

Other Products for Differentiation

- STEMdiff™ Cerebral Organoid Kit

*Flexible products for differentiation can be used for these cell types.

STEMdiff™ Neural System

Differentiation to Neural Progenitor Cells, Neurons and Glia

STEMdiff™ SMADi Neural Induction Kit consists of a serum-free medium and supplement for the highly efficient neural induction of human ES and iPS cells. This kit combines STEMdiff™ Neural Induction Medium with STEMdiff™ SMADi Neural Induction Supplement, which directs differentiation by blocking TGF-b- and BMP-dependent SMAD signaling, resulting in efficient neural induction of even hard-to-differentiate cell lines (data not shown).

Neural progenitor cells (NPCs) can be generated using STEMdiff™ SMADi Neural Induction Kit with either an embryoid body (EB) protocol or monolayer culture protocol. Morphologically distinct neural rosettes are formed in these cultures, indicative of neural induction (Figure 38A). The cultures are enriched for central nervous system (CNS)-type NPCs, which express SOX1, Nestin and PAX6 (Figure 38B,C). STEMdiff™ Neural Rosette Selection Reagent allows rapid and efficient isolation of neural rosettes in order to enrich for CNS-type NPCs.

NPCs generated using STEMdiff™ SMADi Neural Induction Kit can be efficiently expanded and cryopreserved in the serum-free STEMdiff™ Neural Progenitor Medium and STEMdiff™ Neural Progenitor Freezing Medium, respectively. NPCs cultured in STEMdiff™ Neural Progenitor Medium display typical NPC morphology (Figure 38D) and can be efficiently and consistently expanded over multiple passages to generate a large number of cells. Three- to five-fold expansion can be achieved upon each passage (data not shown).

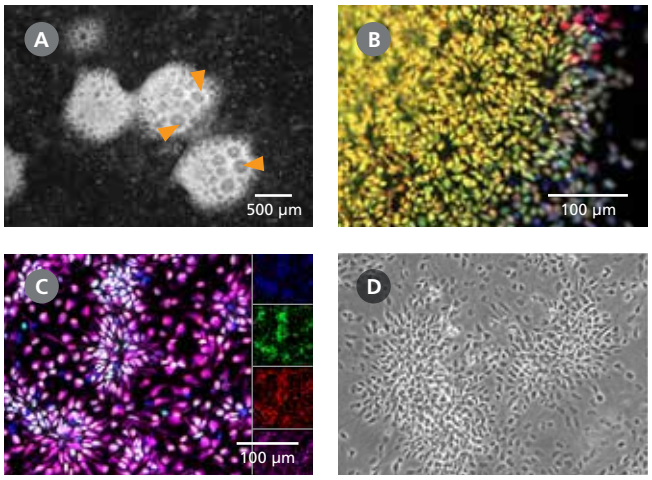


Figure 38. Generation and Expansion of Neural Progenitor Cells Using STEMdiff™ SMADi Neural Induction Kit and STEMdiff™ Neural Progenitor Medium

Starting hPSCs were maintained in mTeSR™1 and differentiated using an EB protocol. (A) Neural rosettes (arrowheads) are clearly visible two days after replating EBs. (B,C) NPCs express CNS-type NPC markers PAX6 (B,C; green), SOX1 (B,C; red) and Nestin (C; purple). Nuclei are counterstained with DAPI. (D) NPCs maintained in STEMdiff™ Neural Progenitor Medium (C) display typical NPC morphology (shown at day 6 of passage 1).

Why Use the STEMdiff™ Neural System?

STREAMLINED WORKFLOW. The STEMdiff™ Neural System offers a complete hPSC-to-neural cell culture workflow.

RAPID NEURAL INDUCTION. STEMdiff™ SMADi Neural Induction Kit.

EFFICIENT EXPANSION OF NPCS. STEMdiff™ Neural Progenitor Medium.

DIFFERENTIATION TO NEURONS AND GLIA. STEMdiff™ Differentiation and Maturation Kits.

HIGH-RECOVERY CRYOPRESERVATION. STEMdiff™ Neural Progenitor Freezing Medium.

NPCs generated using the STEMdiff™ SMADi Neural Induction Kit EB protocol can be differentiated to functional neuronal and glial subtypes using lineage-specific STEMdiff™ Differentiation and Maturation Kits. A mixed population of excitatory and inhibitory forebrain-type (FOXG1-positive) neurons can be generated using the serum-free STEMdiff™ Neuron Differentiation Kit and STEMdiff™ Neuron Maturation Kit. The neurons produced are highly pure, functional and can be maintained long-term in culture (Figure 39A,B). Neurons derived using these kits are electrically active, as evaluated by multielectrode array (data not shown).

Dopaminergic neurons can be generated using the serum-free STEMdiff™ Dopaminergic Neuron Differentiation Kit and STEMdiff™ Dopaminergic Neuron Maturation Kit. The cell population produced contains midbrain-type (FOXA2-, LMX1A- and GIRK2-positive) dopaminergic neurons that can be maintained long-term in culture (Figure 39C). Mature neurons derived using these kits are electrically active, as evaluated by patch-clamp electrophysiology (data not shown).

A highly pure population of astrocytes can be generated using the STEMdiff™ Astrocyte Differentiation Kit and STEMdiff™ Astrocyte Maturation Kit (Figure 39D). These kits are a part of a system that enables generation of astrocytes from hPSCs in as little as 7 weeks; significantly faster than published protocols.

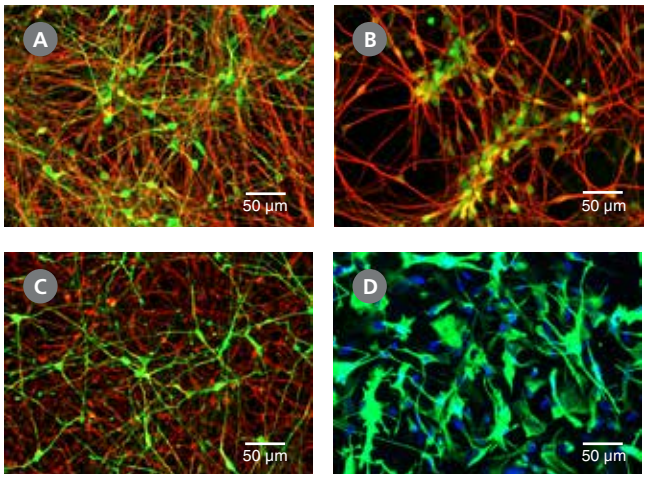


Figure 39. Downstream Differentiation of Neural Progenitor Cells to Neurons, Dopaminergic Neurons and Astrocytes Using STEMdiff™ Differentiation and Maturation Kits

NPCs generated from hPSCs using the STEMdiff™ Neural Induction Medium EB protocol were differentiated and matured to forebrain neurons, dopaminergic neurons and astrocytes using the STEMdiff™ Differentiation and Maturation Kits. (A,B) A mixed population of excitatory and inhibitory neurons was formed after NPCs derived from ES cells (H9 cell line) were cultured in STEMdiff™ Neuron Differentiation Kit for 7 days and STEMdiff™ Neuron Maturation Kit for 21 days. The resulting cultures contain a highly pure population of class III β-tubulin-positive neurons (A,B; red), with a high proportion of GABAergic interneurons (A; GABA, green) (≥ 90% class III β-tubulin-positive neurons; < 10% glial fibrillary acidic protein (GFAP)-positive astrocytes). The neurons generated are forebrain-type (B; FOXG1, green). (C) Dopaminergic neurons were formed when NPCs derived from iPS cells (STiPS-M001 cell line) were cultured in STEMdiff™ Dopaminergic Neuron Differentiation Kit for 13 days and STEMdiff™ Dopaminergic Neuron Maturation Kit for 13 days. The dopaminergic neurons express the neuronal marker class III β-tubulin (red) and the dopaminergic neuronal marker tyrosine hydroxylase (TH; green) (15 - 30% tyrosine hydroxylase (TH)-positive dopaminergic neurons; ≥ 90% class III β-tubulin-positive neurons; < 10% GFAP-positive astrocytes).
(D) Astrocytes with typical star-like morphology were formed when NPCs derived from ES cells (H9 cell line) were cultured in STEMdiff™ Astrocyte Differentiation Kit for 19 - 20 days and STEMdiff™ Astrocyte Maturation Kit for 56 days. The astrocytes express GFAP (green) but not the neuronal marker class III β-tubulin (red) (> 85% GFAP-positive astrocytes; < 15% class III β-tubulin-positive neurons). Nuclei are counterstained with DAPI.

PRODUCT	SIZE	CATALOG #
STEMdiff™ SMADi Neural Induction Kit	1 Kit	08581
STEMdiff™ Neural Induction Medium	250 mL	05835
STEMdiff™ Neural Rosette Selection Reagent	100 mL	05832
STEMdiff™ Neural Progenitor Medium	1 Kit	05833
STEMdiff™ Neuron Differentiation Kit	1 Kit	08500
STEMdiff™ Neuron Maturation Kit	1 Kit	08510
STEMdiff™ Dopaminergic Neuron Differentiation Kit	1 Kit	08520
STEMdiff™ Dopaminergic Neuron Maturation Kit	1 Kit	08530
STEMdiff™ Astrocyte Differentiation Kit	1 Kit	08540
STEMdiff™ Astrocyte Maturation Kit	1 Kit	08550
STEMdiff™ Neural Progenitor Freezing Medium	100 mL	05838
STEMdiff™ Human Neural Progenitor Antibody Panel	1 Kit	69001

Cryopreserved hPSC-Derived Neural Cells

Highly pure cryopreserved hPSC-derived NPCs, mixed neurons, dopaminergic neurons and astrocytes are now available for rapid and reproducible implementation of hPSC-neural models. NPCs can be expanded for over 10 passages in Neural Progenitor Medium 2 without loss of differentiation capacity.

PRODUCT*	SIZE	CATALOG #
Neural Progenitor Medium 2, Basal Medium*	250 mL**	08560
Human PSC (XCL-1)-Derived Neural Progenitor Cells, Male	1 million cells	70901
Human PSC (XCL-4)-Derived Neural Progenitor Cells, Female	1 million cells	70902
Human PSC (XCL-1)-Derived Mixed Neurons, Male	1 million cells	70905
Human PSC (XCL-1)-Derived Dopaminergic Neurons, Male	1 million cells	70909
Human PSC (XCL-1)-Derived Astrocytes, Male	1 million cells	70913

*Only available in select territories

**This medium is only intended to be used with cryopreserved hPSC-derived neural progenitor cells. Kit includes basal medium plus supplements.

STEMdiff™ Cerebral Organoid Kit

Cerebral organoids are three-dimensional in vitro cultures that recapitulate the developmental processes and organization of the developing human brain. They provide a unique, physiologically relevant in vitro model for the study of human neurological development and disease processes that cannot be addressed using animal models. The STEMdiff™ Cerebral Organoid Kit is designed to generate cerebral organoids from human ES and iPS cells.

Using a simple, optimized protocol based on the formulation published by MA Lancaster and JA Knoblich,[14] the cerebral organoids generated with this kit contain multiple layered regions that recapitulate the cortical lamination process observed during in vivo human brain development (Figure 40). For extended periods of organoid culture, the kit components required for organoid maturation are available separately as the STEMdiff™ Cerebral Organoid Maturation Kit.

Why Use the STEMdiff™ Cerebral Organoid Kit?

PHYSIOLOGICAL. Three-dimensional in vitro system recapitulates the developmental processes and organization of the developing brain.

INNOVATIVE. Serum-free hPSC-based model enables the study of development and disease processes.

OPTIMIZED. Formulation is optimized for increased efficiency of organoid formation.

RELIABLE. Rigorous raw material screening and extensive quality control testing ensure reproducibility and minimal lot-to-lot variability.

SIMPLE. Convenient format and easy-to-use protocol.

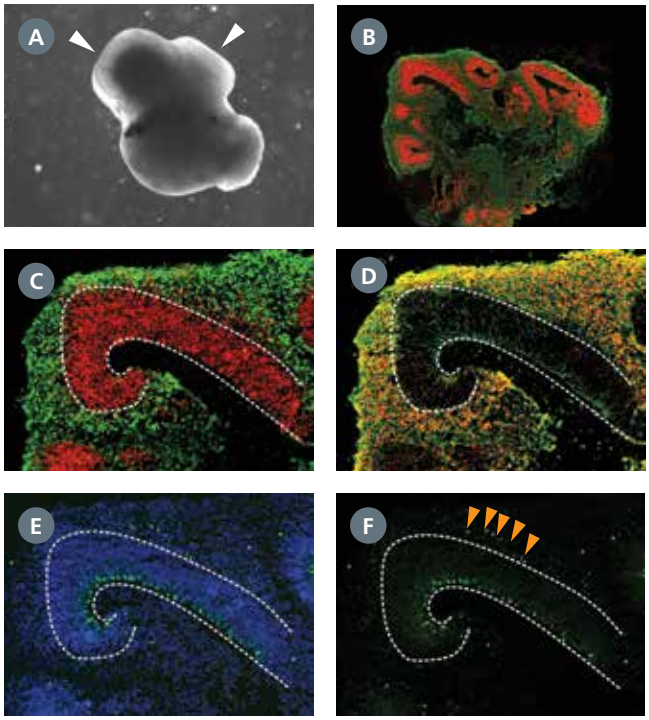


Figure 40. Characterization of Cerebral Organoids Generated Using the STEMdiff™ Cerebral Organoid Kit

(A) A representative phase-contrast image of a whole cerebral organoid at day 40 generated using the STEMdiff™ Cerebral Organoid Kit. Cerebral organoids at this stage are made up of phase-dark structures that may be surrounded by regions of thinner, more translucent structures that display layering (arrowheads). (B) Immunohistological analysis on cryosections of cerebral organoids reveals cortical regions within the organoid labeled by the apical progenitor marker PAX6 (red) and neuronal marker β-tubulin III (green). (C-F) Inset of boxed region from (B). (C) PAX6+ apical progenitors (red, enclosed by dotted line) are localized to a ventricular zone-like region. β-tubulin III+ neurons (green) are adjacent to the ventricular zone. (D) CTIP2, a marker of the developing cortical plate, co-localizes with β-tubulin III+ neurons in a cortical plate-like region. Organization of the layers recapitulates early corticogenesis observed during human brain development. (E) Proliferating progenitor cells labeled by Ki-67 (green) localize along the ventricle, nuclei are counterstained with DAPI (blue). (F) An additional population of Ki-67+ cells is found in an outer subventricular zone-like region (arrowheads). Scale bar = (A) 1 mm, (B) 500 µm and (C-F) 200 µm.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Cerebral Organoid Kit	1 Kit	08570
STEMdiff™ Cerebral Organoid Maturation Kit	1 Kit	08571

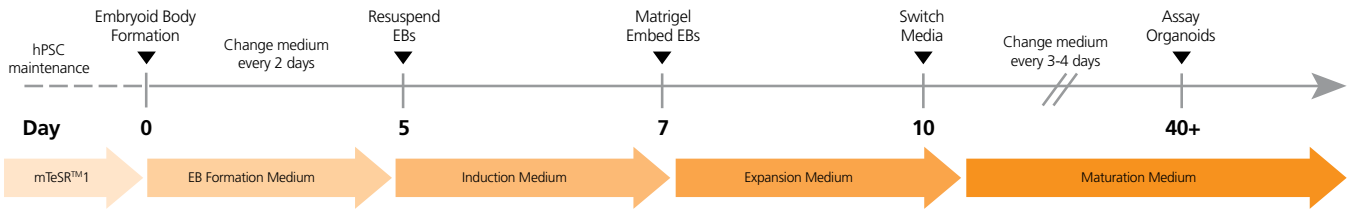


Figure 41. Schematic for the STEMdiff™ Cerebral Organoid Kit

The protocol for generating human cerebral organoids using the STEMdiff™ Cerebral Organoid Kit involves EB formation followed by neural induction and neuroepithelia expansion. Organoids are then matured and maintained.

BrainPhys™ Neuronal Medium

Culture Active Neurons Under Physiological Conditions

Published protocols are available for the generation of many different neuronal subtypes, using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (based on the published B27 formulation[15]) and N2 supplement,[16] and various cytokines and small molecules. Using **BrainPhys™ Neuronal Medium** as the basal medium for hPSC-derived NPC differentation and neuronal maturation will generate a more neurophysiologically active culture that better represents the human brain environment.[17] BrainPhys™ Neuronal Medium may also be used to culture induced neurons derived through lineage conversion of somatic cells (ie. without transitioning through an hPSC intermediate) or through forced Ngn2 expression in hPSCs.[17]

Neurons can be generated efficiently from hPSC-derived NPCs using BrainPhys™ Neuronal Medium and supplements. Patch clamp analysis after 44 days shows that neurons cultured in BrainPhys™ Neuronal Medium are functionally mature and show improved synaptic activity, compared to those cultured in a traditional basal medium (Figure 42). hPSC-derived neurons have been successfully matured in BrainPhys™ Neuronal Medium for up to 126 days in vitro.

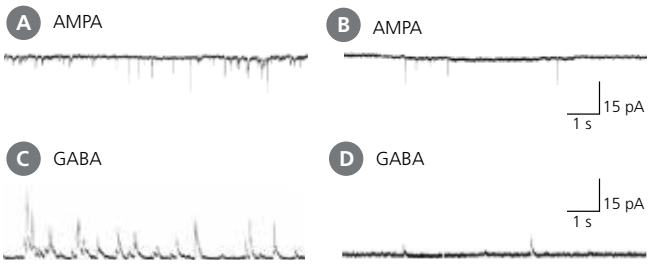


Figure 42. hPSC-Derived Neurons Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity

NPCs were generated from H9 cells using STEMdiff™ Neural Induction Medium in an embryoid body-based protocol. Next, NPCs were cultured for 44 days in vitro in (A,C) BrainPhys™ Neuronal Medium, supplemented with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP and 200 nM ascorbic acid to initiate neuronal differentiation, or (B,D) in DMEM/F12 under the same supplementation conditions. (A,C) Neurons matured in BrainPhys™ Neuronal Medium showed spontaneous excitatory (AMPA-mediated; A) and inhibitory (GABA-mediated; C) synaptic events. The frequency and amplitude of spontaneous synaptic events is consistently greater in neuronal cultures matured in BrainPhys™ Neuronal Medium, compared to neurons plated and matured in DMEM/F12 (B,D). Traces are representative.

Why Use BrainPhys™ Neuronal Medium?

PHYSIOLOGICAL. More representative of the brain's extracellular environment.

ACTIVE. Improved neuronal function and a higher proportion of synaptically active neurons.

STREAMLINED. Perform functional assays without replacing media.

VERSATILE. Supports long-term culture of hPSC- and CNS-derived neurons.

PRODUCT	SIZE	CATALOG #
BrainPhys™ Neuronal Medium	500 mL	05790
BrainPhys™ Without Phenol Red	500 mL	05791
BrainPhys™ Neuronal Medium and SM1 Kit	1 Kit	05792
BrainPhys™ Neuronal Medium N2-A & SM1 Kit	1 Kit	05793
BrainPhys™ hPSC Neuron Kit	1 Kit	05795 COMING SOON
NeuroFluor™ NeuO	0.1 mL	01801

NeuroFluor™ NeuO

Selectively Label Live Neurons

NeuroFluor™ NeuO is a membrane-permeable fluorescent probe that selectively labels primary and pluripotent stem cell-derived neurons in live cultures.[18] Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications.

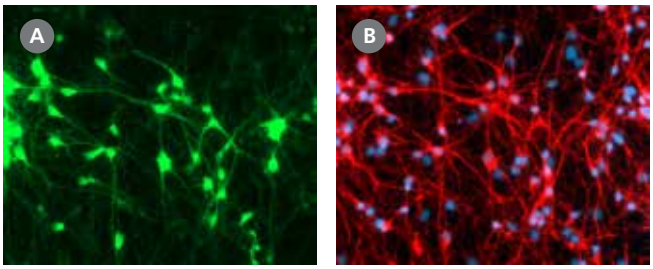


Figure 43. NeuroFluor™ NeuO Selectively Labels hPSC-Derived Neurons

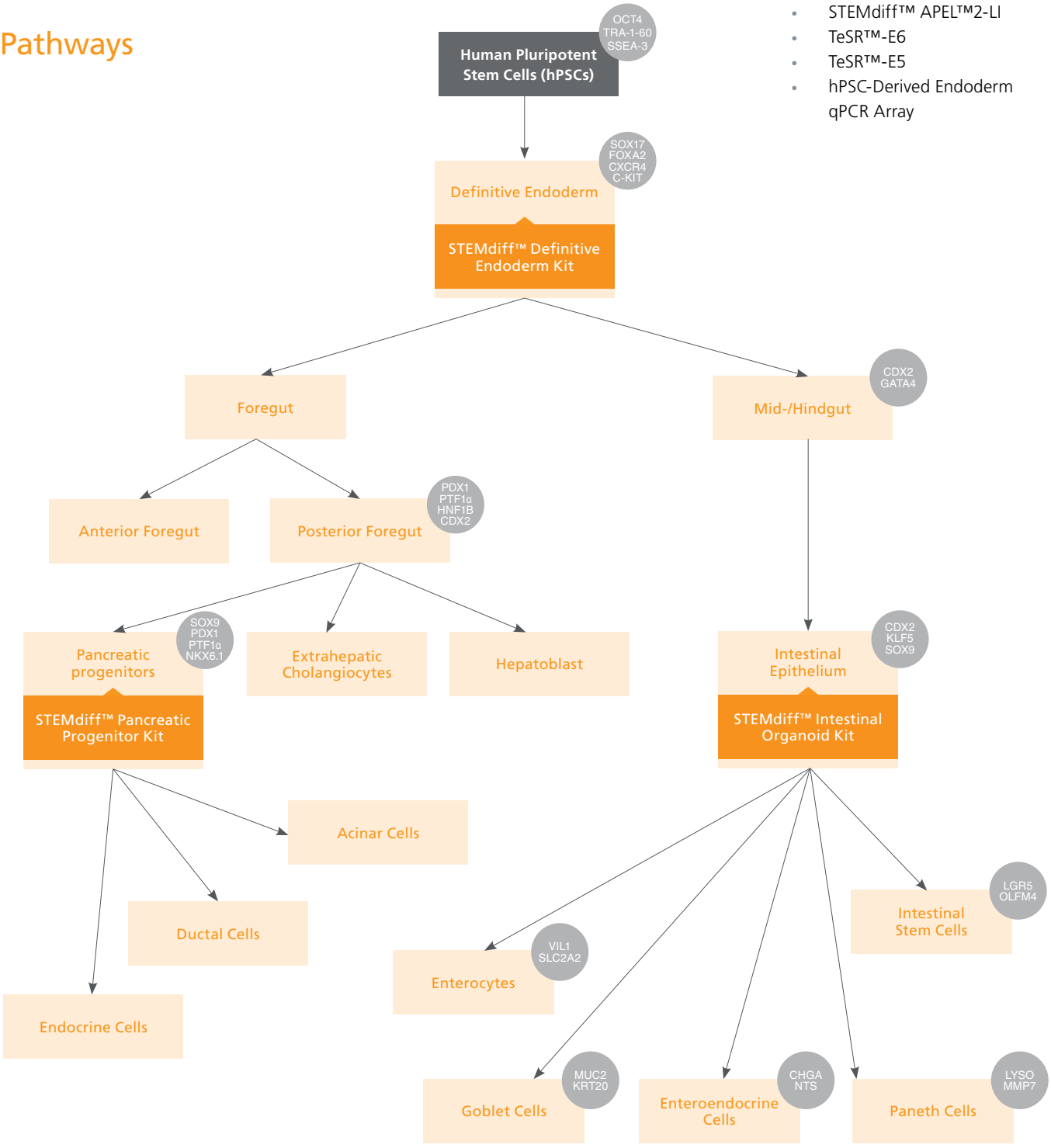
(A) Neuronal precursors generated from hPSC-derived (XCL-1) NPCs were cultured in STEMdiff™ Neuron Maturation Medium. After 18 days of culture, hPSC-derived neurons were labeled with NeuroFluor™ NeuO (green). (B) The same culture was later fixed and immunostained for class III β-tubulin (red). Nuclei are counterstained with DAPI. The images show that NeuroFluor™ NeuO specifically labels class III β-tubulin-positive neurons.

Endoderm Differentiation

Pathways

Flexible Products for Differentiation

- STEMdiff™ APEL™2
- STEMdiff™ APEL™2-LI
- TeSR™-E6
- TeSR™-E5
- hPSC-Derived Endoderm qPCR Array



STEMdiff™ Intestinal Organoid Kit

Directed Differentiation of Intestinal Organoids

hPSC-derived organoids provide a unique platform for studying human tissues in vitro. Organoids provide direct relevance to human tissues while retaining the genotype and phenotype of donor cells.

STEMdiff™ Intestinal Organoid Kit enables the culture of intestinal organoids from ES or iPS cells within 30 days. These organoids incorporate the key cell types and features of the developing intestinal epithelium including the incorporation of some mesenchymal components. Intestinal organoids can be expanded and maintained in culture through passaging, or cryopreserved for future experiments.

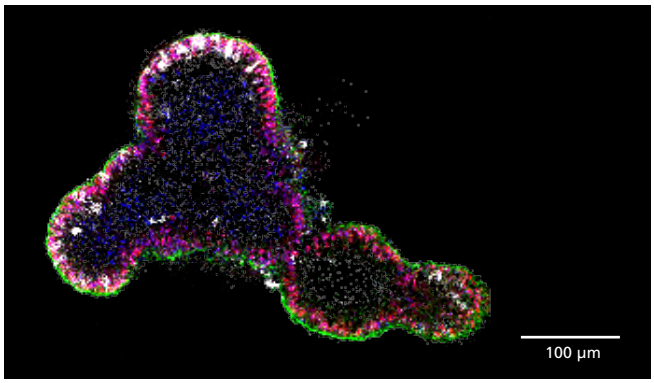


Figure 44. hPSC-Derived Intestinal Organoids Incorporate Features of the Intestinal Epithelium and Mesenchyme

Organoids grown using STEMdiff™ Intestinal Organoid Kit display markers of the intestinal epithelium (EPCAM, CDX2, MUC2). Organoids also exhibit markers for intestinal mesenchyme and intestinal progenitor cells.

Why Use the STEMdiff™ Intestinal Organoid Kit?

RELEVANT. Enables generation of small intestinal organoid cultures that model the developing intestinal epithelium and associated mesenchyme.

ROBUST. Supports efficient differentiation of human ES and iPS cell lines to intestinal organoids.

CONVENIENT. Intestinal organoids can be maintained long-term through passaging or cryopreserved for experimental flexibility.

SERUM-FREE. Optimized formulation for low experimental variability.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Intestinal Organoid Kit	1 Kit	05140
STEMdiff™ Intestinal Organoid Growth Medium	1 Kit	05145

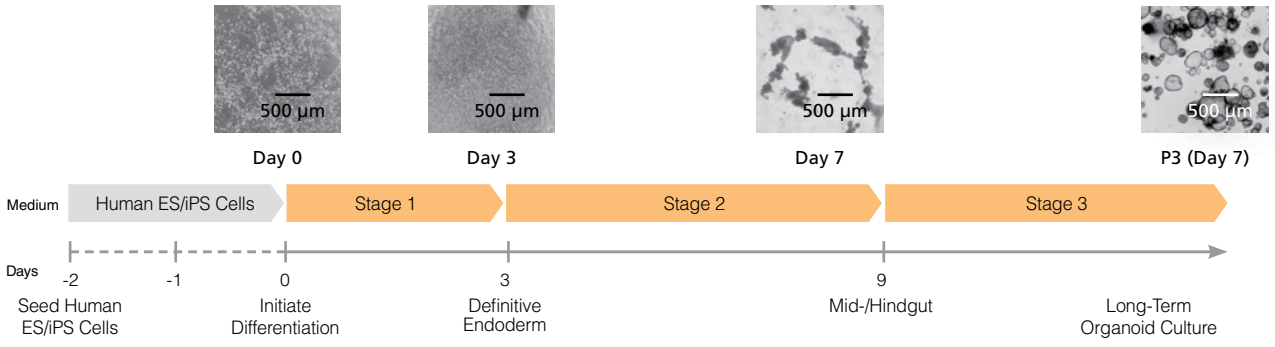


Figure 45. STEMdiff™ Intestinal Organoid Kit Enables Differentiation from hPSCs to Human Intestinal Organoids

hPSC cultures progress through a three-stage differentiation process to generate human intestinal organoids. By day 3 of the protocol, cultures exhibit characteristics typical of definitive endoderm and mid-/hindgut differentiation is initiated. During mid-/hindgut differentiation (days 5 - 7), cells form mid-/hindgut spheroids that are released from the cell monolayer into the culture medium. These spheroids are collected, embedded in extracellular matrix, and cultured in STEMdiff™ Intestinal Organoid Growth Medium to mature into intestinal organoids.

STEMdiff™ Definitive Endoderm Kit

Definitive Endoderm Differentiation

The STEMdiff™ Definitive Endoderm Kit is a serum-free, animal component-free system that enables differentiation of hPSCs to multipotent definitive endoderm cells using a short and simple protocol. This product is available in formulations optimized for use with hPSCs cultured in mTeSR™1 or TeSR™-E8™. Differentiation is efficient and reproducible across multiple ES and iPS cell lines (Figure 46). Futhermore, hPSCs differentiated using either method are highly enriched for definitive endoderm cells, as indicated by co-expression of SOX17, CXCR4 and FOXA2 (Figure 47). Definitive endoderm cells generated with this kit can be further differentiated to multiple downstream endodermal cell types, including hepatic[19] and pancreatic[20] progenitor cells for drug development, toxicity testing, research for development of cell-based therapies, or studying developmental pathways.

Why Use the STEMdiff™ Definitive Endoderm Kit?

ANIMAL COMPONENT-FREE. Serum-free and animal component-free formulation.

OPTIMIZED. Compatible with hPSCs cultured in mTeSR™1 or TeSR™-E8™.

ROBUST. Reproducible differentiation of multiple human ES and iPS cell lines.

MULTIPOTENT. Generate functional endoderm capable of downstream differentiation to multiple lineages.

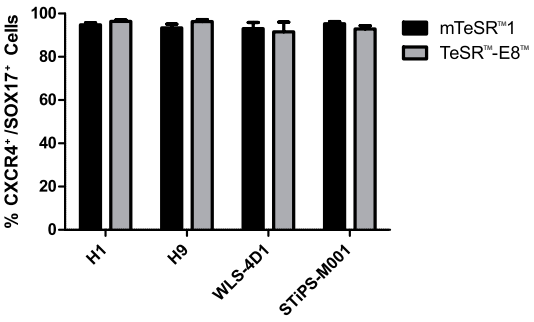


Figure 46. Definitive Endoderm Differentiation is Efficient Across Multiple human ES and iPS Cell Lines, Regardless of hPSC Maintenance Medium

Quantitative analysis of definitive endoderm formation in multiple human ES (H1 and H9) and iPS (WLS-4D1 and STiPS-M001) cell lines as measured by co-expression of CXCR4 and SOX17. Cells maintained in mTeSR™1 medium were differentiated using STEMdiff™ Definitive Endoderm Kit, and cells maintained in TeSR™-E8™ were differentiated using STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized). Data are expressed as the mean percentage of cells expressing both markers. Error bars indicate SEM; n = 4 to 18 per cell line.

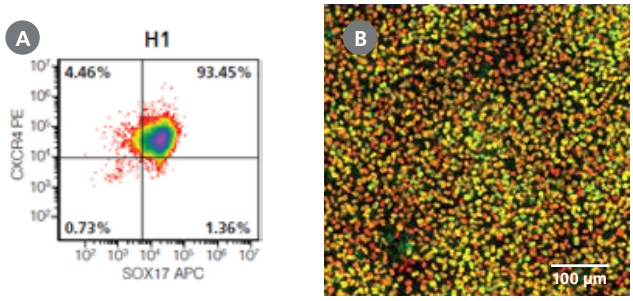


Figure 47. Efficient Expression of Key Definitive Endoderm Markers in hPSCs Differentiated with STEMdiff™ Definitive Endoderm Kit

(A) Representative density plot showing CXCR4 and SOX17 expression in mTeSR™1-cultured H1 ES cells, following 5 days of differentiation. (B) Representative image of FOXA2 (green) and SOX17 (red) in WLS-4D1 iPS cells following 4 days of differentiation. Yellow indicates cells co-expressing FOXA2 and SOX17.

hPSC-Derived Endoderm qPCR Array

The hPSC-Derived Endoderm qPCR Array provides a validated 90-gene assay to characterize definitive endodermal progenitor cells and their differentiated progeny including pancreatic, hepatic and intestinal cells. Housekeeping controls and a synthetic DNA positive control are included. Data analysis is streamlined with our flexible online app (www.stemcell.com/qPCRanalysis).

PRODUCT	SIZE	CATALOG #
STEMdiff™ Definitive Endoderm Kit	1 Kit	05110
STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized)	1 Kit	05115
hPSC-Derived Endoderm qPCR Array	96-well Plate	07531

STEMdiff™ Pancreatic Progenitor Kit

Pancreatic Progenitor Differentiation

The STEMdiff™ Pancreatic Progenitor Kit is a serum-free medium that supports efficient and reproducible generation of pancreatic progenitor cells from hPSCs. The kit is compatible with cells cultured in mTeSR™1 and directs efficient differentiation from multiple hPSC lines through definitive endoderm, primitive gut tube and posterior foregut endoderm before transitioning to pancreatic progenitor cells. The differentiated cells are characterized by expression of key transcription factors including PDX-1, NKX6.1 and NEUROD1, and by the up-regulation of insulin and glucagon (Figures 48,49). The resulting pancreatic progenitor cells can be further differentiated to both exocrine and endocrine cell fates, making them useful research tools for diabetes and β cell maturation, disease modeling and pancreatic cancer.

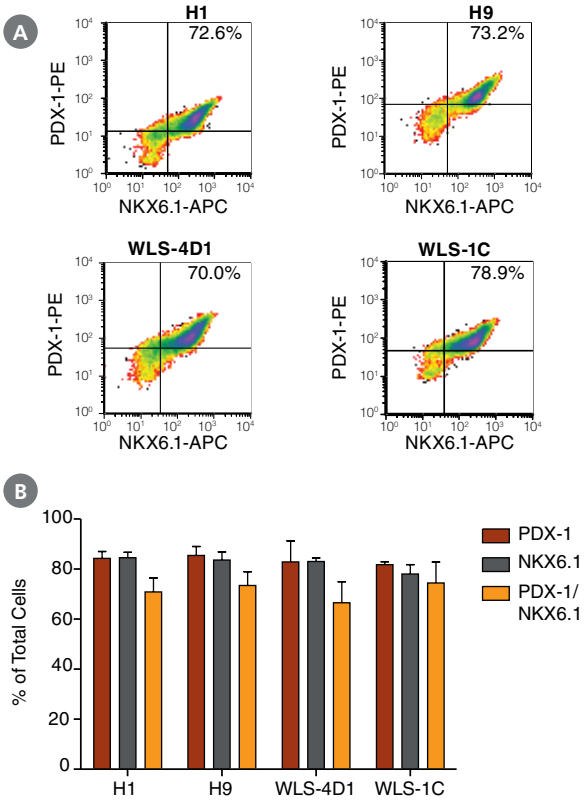


Figure 48. STEMdiff™ Pancreatic Progenitor Kit Functions Efficiently Across Multiple hPSC Lines

PDX-1 and NKX6.1 expression measured in pancreatic progenitor cells derived from four different hPSC lines (H1, H9, WLS-4D1 and WLS-1C). (A) Representative flow cytometry plots for PDX-1 and NKX6.1 expression at the end of Stage 4. (B) Cumulative quantitative data for PDX-1 and NKX6.1 co-expression at the end of Stage 4 (mean ± SD; n = 3 - 5 per cell line). The average efficiency of differentiation ranges from 66.5% to 74.5% depending on the cell line. The efficiency of conversion from definitive endoderm to pancreatic progenitor ranges from 77.3% to 96.3%. In addition, nearly all NKX6.1$^+$ cells co-express PDX-1 as observed in the developing human pancreas.[21]

Why Use the STEMdiff™ Pancreatic Progenitor Kit?

SERUM-FREE. Optimized formulation for low experimental variability.

ROBUST. Reproducible differentiation of multiple human ES and iPS cell lines.

EFFICIENT. Greater than 65% PDX-1$^+$/NKX6.1$^+$ cells in differentiated cultures.

FUNCTIONAL. Pancreatic progenitor cells capable of differentiating toward insulin-producing β-cells or other endocrine and exocrine pancreatic cell fates.

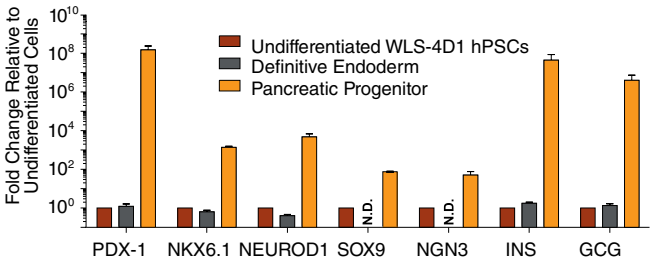


Figure 49. Gene Expression Profile Indicates Transition to Pancreatic Progenitor Cell

Gene expression profile of key transcription factors or hormones (INS: insulin, GCG: glucagon) expressed in pancreatic progenitor cells (mean ± SEM; n = 3 - 7 experiments on WLS-4D1 cells). Expression was first normalized to 18S ribosomal RNA and then to the expression level found in undifferentiated cells. Gene expression is shown for WLS-4D1 cells at the end of Stage 1 (Definitive Endoderm) and at the end of Stage 4 (Pancreatic Progenitor). Expression pattern is consistent with published data.[22] N.D.: Not Determined.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Pancreatic Progenitor Kit	1 Kit	05120

Flexible User-Directed Differentiation

STEMdiff™ APEL™2 and APEL™2-LI

STEMdiff™ APEL™2 Medium is a fully defined, serum-free and animal component-free medium for differentiation of human ES and iPS cells. It is based on the APEL formulation published by Ng et al.[23] 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. Appropriate induction factors must be added before use.

STEMdiff™ APEL™2-LI Medium does not contain growth factors or cytokines, and appropriate induction factors must be added before use. The low insulin content of this medium makes it particularly useful for differentiation to lineages in which insulin is a known inhibitor, such as cardiomyocytes.[24]

Why Use STEMdiff™ APEL™2?

ANIMAL COMPONENT-FREE. Defined, versatile, growth factor-free formulation.

NO LINEAGE BIAS. Unsupplemented medium supports cell survival without lineage bias.

FLEXIBLE DIFFERENTIATION. Can be used to direct differentiation to a variety of cell lineages.

VERSATILE. Can be used in adherent- or EB-based protocols.

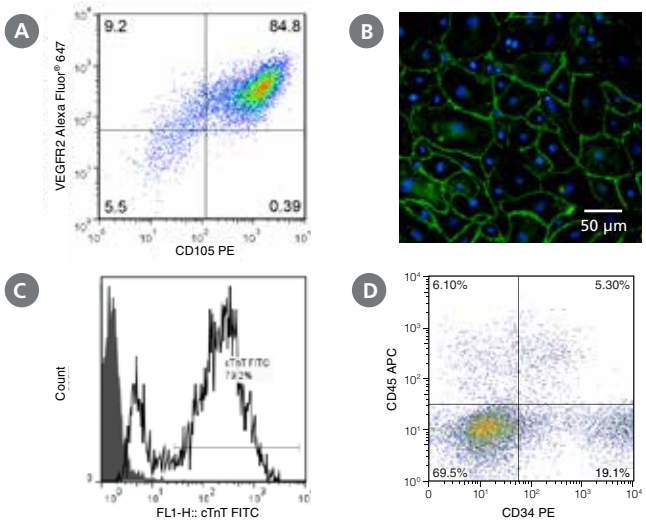


Figure 50. Differentiation of hPSCs to Mesodermal Cell Lineages Using STEMdiff™ APEL™ and STEMdiff™ APEL™-LI Media

(A) Endothelial differentiation of STiPS-F001 human iPS cells using *STEMdiff™ APEL™ medium, based on Tan et al.[25] (B) Immunocytochemistry image of CD31 (green; nuclei shown in blue) in endothelial cells differentiated from H1 cells using STEMdiff™ APEL™ medium. Image courtesy of the Cao Tong lab, University of Singapore. (C) Cardiomyocyte differentiation of WLS-4D1 iPS cells based on Yang et al.,[26] using 6 ng/mL Activin A and 10 ng/mL BMP4, with the following changes: (1) *STEMdiff™ APEL™-LI medium was used as the basal medium; (2) EBs were formed in AggreWell™400 at 1,000 cells/EB; (3) 100 ng/mL Wnt3a was added on days 1 - 4. On day 15, 77.3% (± 1.4%, n = 3) of the cells expressed cardic troponin T (cTnT; solid line). Filled histogram represents unlabeled negative control. (D) Hematopoietic differentiation of H9 cells, based on Ng et al.[23] and Chadwick et al.[27] with the following changes: (1) STEMdiff™ APEL™ medium was used as the basal medium; (2) prior to differentiation, cells were maintained in mTeSR™1 on Matrigel®; (3) differentiation was performed in adherent cell culture on a Matrigel®-coated surface, instead of using an EB-based method. *STEMdiff™ APEL™ and STEMdiff™ APEL™-LI have been updated to STEMdiff™ APEL™2 and STEMdiff™ APEL™2-LI, respectively, and the formulations now do not contain the undefined component, protein-free hybridoma medium.

TeSR™-E5 and TeSR™-E6 Media

TeSR™-E5 and TeSR™-E6 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.

PRODUCT	SIZE	CATALOG #
STEMdiff™ APEL™2 Medium	100 mL	05270
STEMdiff™ APEL™2-LI Medium	100 mL	05271
TeSR™-E5	1 Kit	05916
TeSR™-E6	1 Kit	05946

Differentiated Cells

iPS Cell-Derived iCell® Products

iPS cell-derived iCell® products are manufactured by FUJIFILM Cellular Dynamics, Inc. and distributed by STEMCELL Technologies. These cells can be used as physiologically relevant models in a variety of applications including drug discovery, toxicity testing and regenerative medicine research.

iCell® Cardiomyocytes are a mixture of spontaneous, electrically active atrial, nodal and ventricular cells that exhibit stable cardiac gene expression with proper protein expression and localization. iCell® Cardiac Progenitor Cells are a population of dynamic progenitors that can proliferate and differentiate into mature cardiomyocytes.

iCell® DopaNeurons are a highly pure population of human iPS cell-derived midbrain dopaminergic neurons, ideal for studying neurological disorders including Parkinson's Disease. iCell® GlutaNeurons, an enriched population of human iPSC-derived cortical glutamatergic neurons, provide an excitatory neuronal model for the study of neuronal network development and activity. Both iCell® DopaNeurons and iCell® GlutaNeurons can be cultured with BrainPhys™ Neuronal Medium.

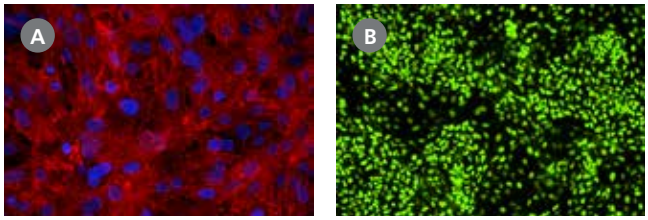


Figure 51. iCell® Cardiomyocytes and iCell® Cardiac Progenitor Cells Maintain Classic Marker Profiles

(A) iCell® Cardiomyocytes exhibit intact myofilaments as indicated by cardiac marker alpha actinin (red) and DAPI (blue) at day 14 post-plating. (B) iCell® Cardiac Progenitor Cells maintain classic $KDR^+/CKIT^-/PDGFR^+$ (not shown) and $NKX2.5^+$ (green) profiles at day 2 post-plating.

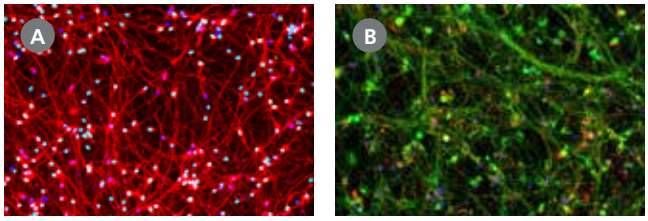


Figure 52. iCell® DopaNeurons and iCell® GlutaNeurons Are Fully Differentiated, Physiologically Relevant Neurons

(A) iCell® DopaNeurons are fully differentiated neurons with floor plate-derived midbrain dopaminergic specificity as demonstrated by FOXA2 (green), TH (red) and Hoechst (blue) immunostaining. (B) iCell® GlutaNeurons are fully differentiated cortical glutamatergic neurons as shown by synaptophysin (red), TUJ-1 (green) and DAPI (blue) immunostaining.

Why Use iCell® Products?

PHYSIOLOGICALLY RELEVANT. iCell® differentiated iPS cells exhibit physiological and functional characteristics similar to those of native human cell types.

REPRODUCIBLE. Each cell type is derived from a single iPS cell line in large batches, ensuring consistency across multiple experiments.

READY-TO-USE. Cells are ready-to-use upon thawing, and kits include optimized media and/or supplements.

iCell® Products

Cells

PRODUCT	SIZE	CATALOG #
iCell® Cardiac Progenitor Cells	≥5 x 10^6 cells	70919
iCell® Mesenchymal Stem Cells	≥1 x 10^6 cells	70922
iCell® Retinal Pigment Epithelial Cells, 01279	≥1 x 10^6 cells	R1102
	≥5 x 10^6 cells	R1101

Kits

PRODUCT	SIZE	CATALOG #
iCell® Cardiomyocytes Kit, 11713	≥1 x 10^6 cells	R1105
	≥4 x 10^6 cells	R1106
iCell® DopaNeurons Kit, 01279	≥1 x 10^6 cells	R1088
	≥5 x 10^6 cells	R1032
iCell® GABANeurons Kit, 01279	≥1 x 10^6 cells	R1084
	≥4 x 10^6 cells	R1011
iCell® GlutaNeurons Kit, 01279	≥1 x 10^6 cells	R1061
	≥6 x 10^6 cells	R1034

FUJIFILM Cellular Dynamics

*Certain products are only available in select territories. Please contact your local Sales representative or Product & Scientific Support at techsupport@stemcell.com for further information.

Small Molecules

Small molecules are increasingly being used as critical tools to understand stem cell biology. Whether used to affect reprogramming, self-renewal, or differentiation, the right small molecule can transform a research project. STEMCELL Technologies offers small molecules that are being widely used in high impact research to target the key pathways in stem cell biology.

Most Popular Small Molecules

MOLECULE	PATHWAY/ TARGET	APPLICATIONS	CATALOG #
Y-27632	RHO/ROCK pathway inhibitor Inhibits ROCK	Maintenance	72302
CHIR99021	WNT pathway activator Inhibits GSK3	Reprogramming, Maintenance, Differentiation	72052
IWP-2	WNT pathway inhibitor Inhibits Porcupine	Maintenance, Differentiation	72122
LDN193189	BMP pathway inhibitor Inhibits ALK2, ALK3, ALK6	Differentiation	72146
SB431542	TGF-β inhibitor Inhibits ALK5, ALK4, ALK7	Reprogramming, Differentiation, Maintenance	72232
Thiazovivin	RHO/ROCK pathway inhibitor Inhibits ROCK	Maintenance, Reprogramming	72252
PD0325901	MEK/ERK pathway inhibitor Inhibits MEK	Reprogramming, Maintenance	72182
Purmorphamine	Hedgehog pathway activator Activates Smoothened	Differentiation	72202
DAPT	Notch pathway inhibitor Inhibits γ-secretase	Differentiation, Maintenance	72082
Prostaglandin E2	Prostanoid pathway activator Activates prostaglandin receptors EP1, EP2, EP3 and EP4	Differentiation, Maintenance	72192
A 83-01	TGF-β inhibitor Inhibits ALK5, ALK4, ALK7	Reprogramming, Maintenance	72022
SU5402	Inhibits VEGFR2, FGFR1, and PDGFRβ	Maintenance	73912

For a complete listing and more details on the small molecules available, and to see how they are being used in high-impact studies, visit **www.stemcell.com/smallmolecules**.

Cytokines

Cytokines are commonly used tools in lineage-specific differentiation protocols, as well as for self-renewal of hPSCs. For a complete listing of cytokines available, including animal-component free versions, please visit **www.stemcell.com/cytokines**.

Most Popular Cytokines

PRODUCT	CATALOG #
Activin A	78001
bFGF	78003
BMP-4	02524
Flt3/Flk-2 Ligand	78009
LIF	78055 (human) 78056 (mouse)
Noggin	78060
SCF	78062
TGF-β1	78067
VEGF-165	78073
VEGF-121	78127

AggreWell™ Plates

Reproducible Production of Uniform Embryoid Bodies

Many hPSC differentiation protocols begin with the formation of three-dimensional aggregates of cells called embryoid bodies (EBs). Conventional EB formation methods[28] result in EBs that are heterogeneous in size and shape (Figure 53A), leading to inefficient and uncontrolled differentiation.[29]

AggreWell™ plates provide an easy and standardized approach to the production of EBs. Each well contains microwells of defined size, making it easy to produce large numbers of highly uniform EBs (Figure 53B) and to ensure reproducibility of differentiation experiments.[30]

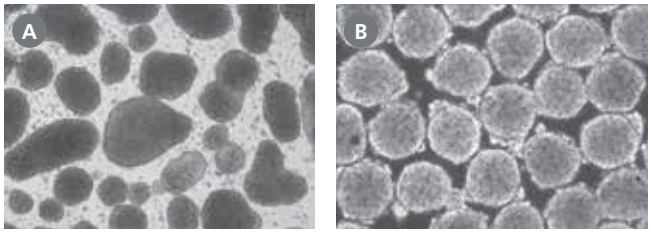


Figure 53. AggreWell™ Plates Are Used to Generate Uniform EBs

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

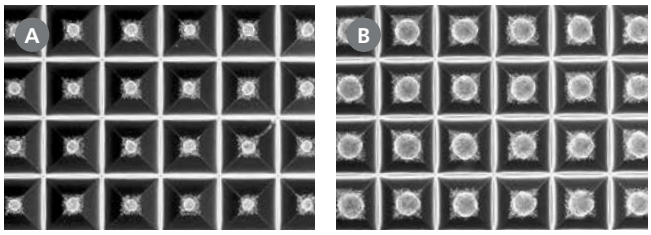


Figure 54. The Size of EBs Can be Controlled in AggreWell™

Starting from a single cell suspension, hPSCs form EBs after 24 hours in AggreWell™. The size of the EB can be easily modified by adjusting the seeding density. Shown are EBs in AggreWell™400 (A) 250 cells per microwell and (B) 1000 cells per microwell.

Why Use AggreWell™?

EASY TO USE. Simple EB generation.

REPRODUCIBLE. Produce large numbers of uniformly sized EBs.

CONTROL OF EB SIZE. 50 to 20,000 cells per EB.

CONSISTENCY. Reduces variability in differentiation protocols.

HIGH YIELD. Up to 7000 EBs per well.

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	~ 7000 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	~1800 per well	34821/34825

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

Antibodies

For Human Pluripotent Stem Cells and Differentiated Cells

STEMCELL Technologies' 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.

Most Popular hPSC-Related Antibodies

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**.

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