ThermoFisher SCIENTIFIC

PLURIPOTENT STEM CELL PRODUCT GUIDE

Key products and services for PSC research

Make the connection

gibco

The pluripotent stem cell workflow

Somatic and progenitor cells

The reprogramming process starts with human somatic cells

Human somatic cells
Blood (CD34+)
Epithelial (corneal, mammary)
Fibroblasts (HDFn, HDFa)
Keratinocytes (HEKn, HEKa)

Gibco media
StemPro-34 SFM
Advanced DMEM
DMEM/F-12
KSFM

Cell culture plastics
Nunc flasks, dishes, and plates

Reprogram

Reprogram adult cells, such as blood cells, back to a pluripotent state to become induced pluripotent stem cells (iPSCs)

Virus
CytoTune-iPS 2.0 Sendai Reprogramming Kit

DNA
Episomal vectors
Epi5 vectors

Transfection reagents
Lipofectamine 3000 Transfection Reagent
Lipofectamine MessengerMAX Transfection Reagent

Cell culture plastics
Nunclon Delta and extracellular matrix (ECM)-coated plates

thermofisher.com/reprogramming

Culture

Expand and maintain iPSCs, which requires careful attention to culture conditions

Media
StemScale PSC Suspension Medium
StemFlex Medium
Essential 8 Medium
Essential 8 Flex Medium
KnockOut Serum Replacement

Supplements and reagents
RevitaCell Supplement
PSC Cryopreservation Kit

Matrices
Geltrex matrix
Vitronectin (VTN-N) Recombinant Human Protein
rhLaminin-521

Cell culture plastics
Nunclon Delta plates and dishes with a matrix coating (2D)
Nunclon Sphera plates and dishes (3D)

thermofisher.com/pscculture

Engineer

Engineer iPSCs to analyze disease pathways, better understand gene and protein expression, and study differentiation

Cell culture plastics
Nunclon Delta and ECM-coated plates

Transfection
Lipofectamine Stem Transfection Reagent
Lipofectamine 3000 Transfection Reagent
Lipofectamine CRISPRMAX Transfection Reagent
Lipofectamine MessengerMAX Transfection Reagent
Neon Transfection System

thermofisher.com/transfection

Genome editing
TrueTag Donor DNA Kits
TrueCut Cas9 Protein v2
TrueGuide Synthetic gRNAs

thermofisher.com/genomeedit

Differentiate

Differentiate reprogrammed cells into specific cell types

Ectoderm
B-27 Supplement
CultureOne Supplement
PSC Dopaminergic Neuron Differentiation Kit
PSC Neural Induction Medium

Mesoderm
PSC Cardiomyocyte Differentiation Kit

Endoderm
PSC Definitive Endoderm Induction Kit

Media
Essential 6 Medium

Cell culture plastics
Nunclon Delta and ECM-coated plates and dishes (monolayer culture)
Nunclon Sphera plates and dishes (spheroid and organoid cultures)
Growth factors

thermofisher.com/differentiation

Characterize

Analyze stem cells and their derivatives using a wide range of cellular and molecular characterization methods, as validation is critical in iPSC research.

Tools
KaryoStat and KaryoStat HD Assays
PluriTest-compatible PrimeView Global Gene Expression Profile Assays
TaqMan hPSC Scorecard Panel
Invitrogen immunocytochemistry and live staining kits
Invitrogen primary and secondary antibodies
GeneArt Genomic Cleavage Detection Kits

Instrumentation
CellInsight CX7 High Content Analysis Platform
Attune NxT Flow Cytometer
EVOS cell imaging systems

Cell culture plastics
Nunc Lab-Tek and Lab-Tek II Chamber Slides and Chambered Coverglasses
Nunc Glass Bottom Dishes
Nunc Optical Bottom Plates

thermofisher.com/detectpsc

Ready to transition your stem cell therapy to the clinic?

See **page 54** for complementary Gibco™ Cell Therapy Systems (CTS™) formulations, which are designed to enable clinical and commercial GMP cell therapy manufacturing.

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Supporting research from somatic to differentiated cells

Human pluripotent stem cell research holds tremendous potential in the areas of developmental biology, disease modeling, and cell therapy. The scientists at Thermo Fisher Scientific focus on developing tools to manipulate pluripotent stem cells (PSCs) using novel approaches for reprogramming, long-term culture and propagation, and characterization of these cells.

The wide range of products and services offered by Thermo Fisher Scientific helps simplify your workflow and provides you with more control, enabling faster, more efficient systems.

Somatic and progenitor cells—the starting point for stem cell research

Whether the end goal of your experiment is to understand the basic biology of cells or to reprogram them to eventually differentiate them into a terminal lineage, having the best starting material is critical for downstream applications. We offer a comprehensive range of high-quality Gibco™ cells and expansion media, giving you the ability to advance your cells to your next research step.

Choose your cell type of interest and see more about products and services at **thermofisher.com/stemcells**

Support resources

- Request the Mesenchymal Stem Cell (MSC) Sourcebook, a product reference guide supporting your MSC and adipose-derived stem cell (ADSC) workflow, at **thermofisher.com/mscbook**
- View stem cell protocols for expanding somatic cells at **thermofisher.com/stemcellprotocols**

Table 1. Somatic and progenitor cell media overview.

Cell type	ADSC*	MSC*	CD34⁺ and PBMC*	PBMC	T cell	NSC*	Human fibroblast
Human adult stem and primary cells	StemPro Human Adipose-Derived Stem Cells	StemPro BM Mesenchymal Stem Cells	StemPro CD34⁺ Cell Kit	NA	NA	NA	Human Dermal Fibroblasts, neonatal or adult
Recommended culture media	StemPro Human Adipose-Derived Stem Cell Kit	StemPro MSC SFM XenoFree	StemPro-34 SFM	StemPro-34 SFM	CTS OpTmizer T Cell Expansion SFM** CTS Immune Cell SR	StemPro NSC SFM	DMEM, high glucose; GlutaMAX Supplement, pyruvate; and FBS, embryonic stem cell–qualified
GMP-compliant	Media	Media and cells	Media	Media	Media	Media	Media
Application	Reduces doubling times and variability of ADSCs	Xeno-free medium for human ADSC and MSC expansion	Supports CD34⁺ cell expansion and CytoTune reprogramming from cord blood and bone marrow	Serum-free medium supports PBMC expansion and reprogramming	Medium for T cell expansion	Serum-free medium for NSC expansion	Culture of fibroblasts prior to reprogramming with CytoTune 2.0 kit
Antibodies	Find antibodies for all stem cell targets at **thermofisher.com/antibodies**						

* ADSC = adipose-derived stem cell, MSC = mesenchymal stem cell, PBMC = peripheral blood mononuclear cell, NSC = neural stem cell.

** For human *ex vivo* tissue and cell culture processing applications. CAUTION: When used as a medical device, Federal Law restricts this device to sale by or on the order of a physician.


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REF A16517
LOT L2180063
CytoTune™-iPS 2.0
Sendai Reprogramming Kit
See COA
-65 °C
-68 °C
Read SDS
Made in Japan
For Research
Use Only
IMPORTANT: Titer varies with each lot.
Go to www.thermofisher.com/cytotune to search CoA to find lot specific volumes.
7335 Executive Way • Frederick MD 21704 USA • www.thermofisher.com

Reprogramming

Reprogramming somatic cells to induced PSCs (iPSCs) is a critical and potentially time-intensive step in stem cell research. We offer choices in integration-free reprogramming technologies and services to support your research goals. In addition to these technologies and services, characterization options for PSCs include products for cell identity confirmation pre- and post-reprogramming, and detection of pluripotency in expanding embryonic stem cells (ESCs) and iPSCs.

Find the best solution for your reprogramming experiment at **thermofisher.com/reprogramming**

Support resources

- View cell reprogramming protocols at **thermofisher.com/pscprotocols**
- Access technical resources for CytoTune-iPS kits at **thermofisher.com/cytotuneresources**

Table 2. Nonintegrating reprogramming products and services overview.

Product name	Episomal iPSC Reprogramming Vectors*	Epi5 Episomal iPSC Reprogramming Kit**	CytoTune-iPS 2.0 Sendai Reprogramming Kit	CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit
Applications	Viral-free iPSC generation from normal and diseased cell types	Viral-free iPSC generation from normal and diseased cell types	Highest-efficiency, integration-free reprogramming system	Integration-free iPSCs for clinical and translational research
Reprogramming efficiency	0.002–0.08%	0.04–0.3%	0.02–2%	0.01–0.6%
Factors expressed from genes utilized	Thomson and Yamanaka factors	Yamanaka factors + Lin28	Yamanaka factors	Yamanaka factors (L-myc replaces c-myc)
Blood reprogramming	Yes (with Neon system only)	Yes (with Neon system only)	Yes	Yes
Delivery method	Neon electroporation	Neon electroporation or Lipofectamine 3000 Transfection Reagent	Transduction	Transduction

* Commercialized in partnership with Cellular Dynamics International.

** Designed by CiRA/Dr. K. Okita of CiRA/the Yamanaka Lab at CiRA/Kyoto University.

Need help reprogramming your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals.
See the section starting on **page 58** for all of our stem cell services.

CytoTune-iPS 2.0 Sendai Reprogramming Kit

Highest success rate among nonintegrating reprogramming technologies

The Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit contains three vectors and requires only one overnight incubation compared to multiple days of transductions required for mRNA reprogramming. The kit contains a polycistronic vector, which offers high reprogramming efficiency of up to 2% (Figure 1). This polycistronic vector has a different backbone containing temperature-sensitive mutations in polymerase-related genes, which helps to clear the virus faster after reprogramming and causes less cytotoxicity to the cells.

Why CytoTune-iPS Sendai Reprogramming?

- High success rates for both fibroblast and blood reprogramming [1]
- Scalable cell line generation with minimal hands-on time
- Rapid clearance of RNA vectors
- Transition from research to clinical applications with minimal effort using Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit **(page 57)**

Get more information on CytoTune reprogramming at **thermofisher.com/cytotune**

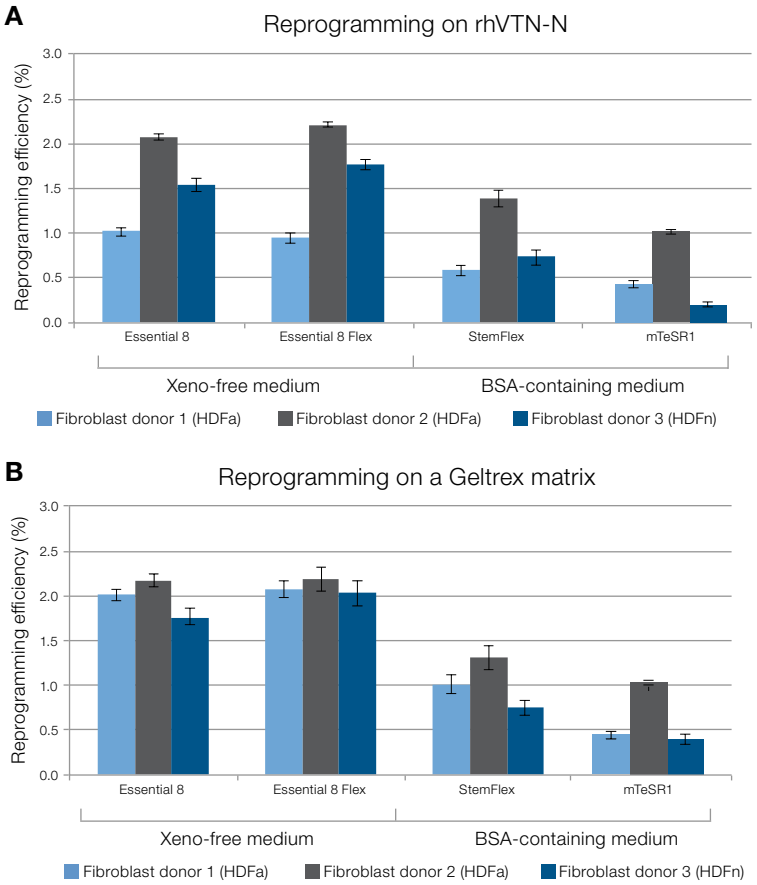


Figure 1. Reprogramming efficiency of human dermal fibroblasts using feeder-free medium conditions on Gibco™ Geltrex™ and rhVTN-N substrates. Fibroblasts from three donors, two adult and one neonatal, were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 7, 50,000 viable cells were transferred per well of a 6-well plate onto either **(A)** rhVTN-N or **(B)** Geltrex matrices, and from day 8 onward, were either fed daily with Gibco™ Essential 8™ Medium or mTeSR™1 Medium, or every other day with Gibco™ Essential 8™ Flex Medium or StemFlex™ Medium. On day 21, alkaline phosphatase staining was completed, and colony counting was performed using the IncuCyte™ ZOOM System to determine the reprogramming efficiency (percentage reprogramming efficiency = colonies counted/50,000 viable cells seeded x 100; n = 3 per condition). BSA = bovine serum albumin

Table 3. Somatic cell types that have been successfully reprogrammed with CytoTune kits.

Human			Nonhuman
Adult and neonatal dermal fibroblasts	Dental pulp stem cells	T cells	Canine fibroblasts
Amniotic fluid MSCs	Mammary epithelial cells	Umbilical vein epithelial cells	Chimpanzee peripheral mononuclear cells
Cardiac fibroblasts	Nasal epithelial cells	Epithelial cells in urine	Macaque dermal fibroblasts
$CD34^+$ blood cells	PBMCs		Mouse embryonic fibroblasts
Conjunctival cells	Skeletal myoblasts		Northern white rhinoceros fibroblasts
			Rhesus monkey dermal fibroblasts

Find publications citing Sendai virus for iPSC generation at **thermofisher.com/sendaipubs**

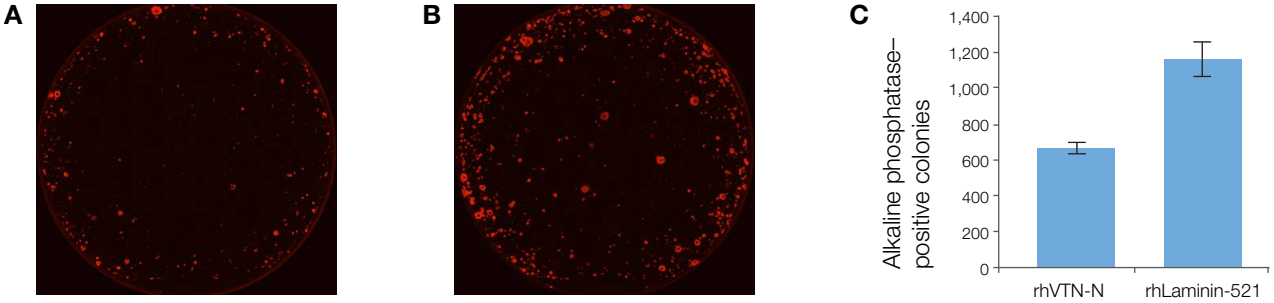


Figure 2. Improvement of feeder-free reprogramming efficiency using alternative matrices or addition of Gibco™ RevitaCell™ Supplement on day 7 transfer. Feeder-free reprogramming of Gibco™ Human Dermal Fibroblasts, neonatal (HDFn) was completed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at a multiplicity of infection (MOI) of 5:5:3. On day 7 post-transduction, reprogrammed fibroblasts were transferred to a rhVTN-N matrix in growth medium in **(A)** the absence and **(B)** the presence of RevitaCell Supplement for 24 hours post-transfer, followed by daily feeding with Essential 8 Medium alone. **(C)** Alternatively, cells can be transferred to rhLaminin-521 on day 7 to boost efficiency of reprogramming.

Need even better reprogramming efficiency?

Supplement PSC culture media on day 7 of reprogramming with RevitaCell Supplement or, alternatively, transfer cells to a rhLaminin-521 matrix on day 7 of reprogramming (Figure 2).

Characterization tools

Alkaline Phosphatase Live Stain

Invitrogen™ Alkaline Phosphatase Live Stain is used for stem cell imaging that allows you to differentially stain PSCs. The dye is a cell-permeant fluorescent substrate for alkaline phosphatase (AP) that is nontoxic to cells, diffusing away over the course of 2 hours.

Find out more at **thermofisher.com/aplivestain**

Live-cell immunostaining

More specific cell staining can be achieved using antibodies against established markers. Surface proteins such as positive and negative PSC markers are particularly useful.

Find out more at **thermofisher.com/pscimmunokits**


gibco
1X
Nutrient Mix
Essential 8® Flex Basal Medium
RevitaCell
PSC Cryomedium
StemScale™
PSC Suspension Basal Medium

Pluripotent stem cell culture

We recognize and understand the preparation that goes into generating PSCs. We know that PSC research requires careful attention to culture conditions to enable successful results. From media and reagents for feeder-dependent and feeder-free systems to those designed to support cell therapy research, Gibco™ products deliver culture with confidence.

Find the right PSC media for your research at **thermofisher.com/pscculture**

Support resources

- View stem cell culture protocols at **thermofisher.com/pscprotocols**
- Access PSC culture how-to videos at **thermofisher.com/stemcellhowto**

Table 4. Gibco™ media systems for PSC culture.

		Feeder-dependent culture	Feeder-free culture				Suspension culture
Medium		**KnockOut Serum Replacement**	**StemFlex Medium**	**Essential 8 Medium**	**Essential 8 Flex Medium**	**CTS Essential 8 Medium**	**StemScale PSC Suspension Medium**
Ideal for		Feeder-based human PSC culture, reprogramming, gene editing, and differentiation	Robust maintenance of PSC cultures, especially when using difficult cell lines or performing single-cell passaging and gene editing	Routine and consistent PSC expansion and maintenance	Routine PSC culture with flexible feeding schedule	Translational or clinical research applications	Robust expansion of PSCs in suspension culture
Defined		Animal-origin components (BSA)	Animal-origin components (BSA)	Xeno-free	Xeno-free	Components not directly derived from animals	Animal-origin components
Recommended cell types		Human PSCs	Human PSCs	Human PSCs	Human PSCs	Human PSCs	Human PSCs
Weekend-free feeding schedule		No	Yes	Yes, Essential 8 Flex Medium	Yes	No	Yes
Genome editing		Fair	Best	Fair	Fair	Fair	NA
Robustness		Good	Best	Good	Good	Good	Best
Recommended matrix		mouse embryonic fibroblasts and attachment factor	Geltrex matrix rhLaminin-521	Vitronectin (VTN-N) Recombinant Human Protein rhLaminin-521	Vitronectin (VTN-N) Recombinant Human Protein rhLaminin-521	CTS Vitronectin (VTN-N) Recombinant Human Protein rhLaminin-521	NA
Recommended level of dissociation and passaging reagent	**Clump**	Collagenase IV	Versene Solution	Versene Solution	Versene Solution	CTS Versene Solution	Not recommended
	Small cluster	NA	StemPro Accutase Cell Dissociation Reagent, RevitaCell Supplement helpful but not required	StemPro Accutase Cell Dissociation Reagent with addition of RevitaCell Supplement recommended during the first 18–24 hours post-passage for improved recovery	StemPro Accutase Cell Dissociation Reagent with RevitaCell Supplement	StemPro Accutase Cell Dissociation Reagent wth addition of CTS RevitaCell Supplement recommended during the first 18–24 hours post-passage for improved recovery	StemPro Accutase Cell Dissociation Reagent
	Single cell	NA	TrypLE Select Enzyme, RevitaCell Supplement helpful but not required during recovery if using rhLaminin-521	TrypLE Select Enzyme with RevitaCell Supplement added to the medium during the first 18–24 hours post-passage for improved recovery	TrypLE Select Enzyme with RevitaCell Supplement	CTS TrypLE Select Enzyme with CTS RevitaCell Supplement added to the medium during the first 18–24 hours post-passage for improved recovery	TrypLE Select Enzyme with DNase or StemPro Accutase Cell Dissociation Reagent

Need help growing and banking your iPSC line?

The team of dedicated stem cell scientists at Thermo Fisher Scientific can help you create your iPSC banks using the latest Gibco media. See the section starting on **page 58** for all of our stem cell services.

StemFlex Medium

Enhanced flexibility and superior performance in today's stem cell applications

StemFlex Medium supports the robust expansion of feeder-free PSCs and is optimized to deliver superior performance in novel applications, including single-cell passaging, gene editing, and reprogramming. Its unique formulation offers the convenience of a flexible feeding schedule (including weekend-free options) and the ability to choose the matrix and passaging reagent that best suits specific applications. StemFlex Medium maintains cells' ability to differentiate into all three germ layers and enables the long-term feeder-free culture of PSCs without karyotypic abnormalities, for up to 50 passages (Figures 3 and 6).

Why StemFlex Medium?

- Superior performance in gene editing, single-cell passaging, and other stressful applications **(see pages 34–37)**
- Easy adaptation from other media systems with no optimization or additional reagents required (Figure 4)
- Use when you need a robust formulation for everyday culture
- Great for difficult cell lines

Find out more about StemFlex Medium at **thermofisher.com/stemflex**

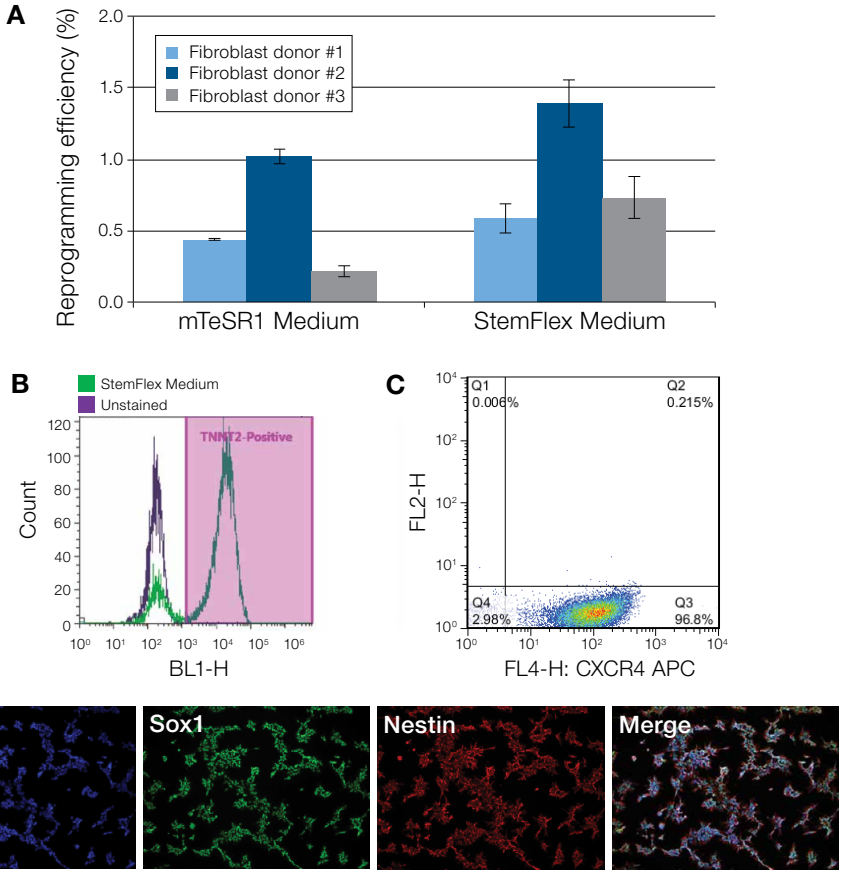


Figure 3. StemFlex Medium provides a robust formulation that can be applied across the entire PSC workflow—from somatic cell reprogramming (A) through downstream differentiation (B–D). When compared to traditional feeder-free media like mTeSR1, StemFlex Medium delivers superior performance across the workflow with the added benefit of enhanced flexibility. Following up to 50 passages on a weekend-free feeding schedule, PSCs expanded in StemFlex Medium maintain the ability to differentiate into: **(B)** mesoderm, as shown by expression of TNNT2 following differentiation using the Gibco™ PSC Cardiomyocyte Differentiation Kit; **(C)** endoderm, as shown by the $CXCR4^+$, $PDGFR\alpha^-$ phenotype following differentiation using the Gibco™ PSC Definitive Endoderm Induction Kit; and **(D)** ectoderm, as shown by expression of Sox1 and nestin following differentiation using Gibco™ PSC Neural Induction Medium.

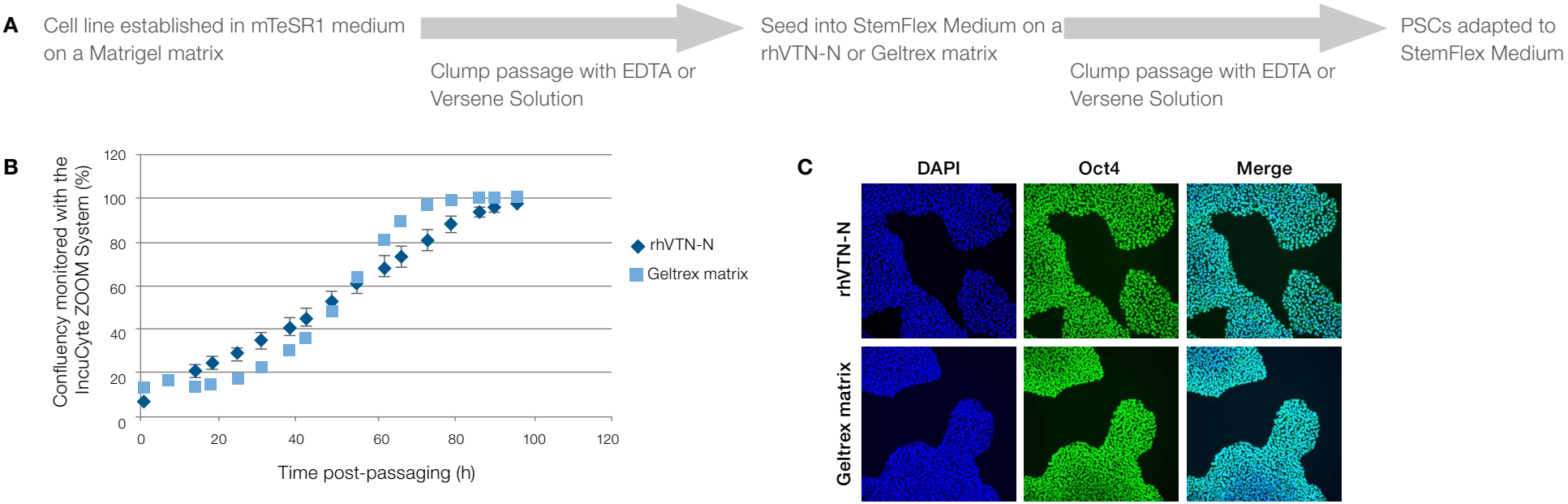


Figure 4. Adaptation of PSCs from mTeSR1 Medium on a Matrigel™ matrix to StemFlex Medium on a Geltrex matrix or rhVTN-N substrate. (A) Existing PSC lines in mTeSR1 Medium can be easily transitioned to StemFlex Medium following a minimum of two passages for full adaptation. **(B, C)** Cells grow well and exhibit high expression of Oct4 whether on a rhVTN-N substrate or a Geltrex matrix.

Pro tips

- Allow at least two passages in StemFlex Medium for full adaptation (Figure 4)
- For frozen vials, thaw into original medium and substrate, then transition into StemFlex Medium
  - Alternatively, cryopreserved PSC stocks that easily recover from cryopreservation can be thawed directly into StemFlex Medium; however, some cell lines may benefit from one passage in the original culture system prior to transition

Weekend-free feeding with Gibco PSC media

Eliminate daily feeding schedules with confidence

Traditional methods of culturing PSCs require that the cultures be fed daily due to the heat sensitivity of key factors such as FGF-2. Typically, the occasional weekend off is allowed by adjusting the protocol and hoping there is minimal impact to the pluripotency of the cultures from skipping a few days. To address this weakness in the PSC culture workflow, we have created two unique formulations, Essential 8 Flex Medium and StemFlex Medium.

Essential 8 Flex and StemFlex media:

- Contain wild-type FGF-2
- Maintain pluripotency more consistently by stabilizing heat-sensitive components like FGF-2 (Figures 5 and 6)
- Allow for skipping up to 2 consecutive days for a total of 3 “feeding-free” days in a week (Figure 7)
- Reduce media consumption by up to 30% and thus also reduce costs compared to traditional feeder-free media

Find out more about these media at **thermofisher.com/stemflex** and **thermofisher.com/essential8flex**

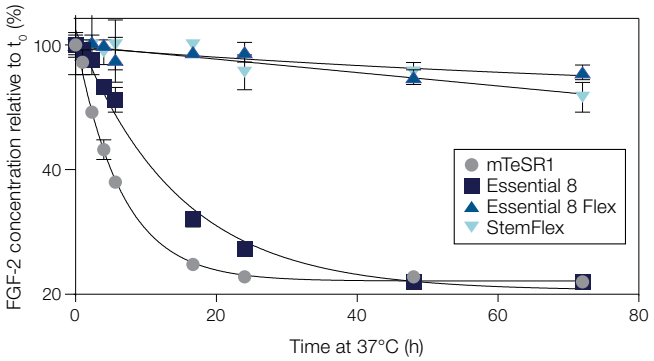


Figure 5. Stability of FGF-2 in StemFlex and Essential 8 Flex media. Both StemFlex and Essential 8 Flex media provide prolonged FGF-2 stability when incubated at 37°C, 5% CO_2, allowing for flexible feeding schedules, including the weekend-free option—eliminating daily feeding requirements.

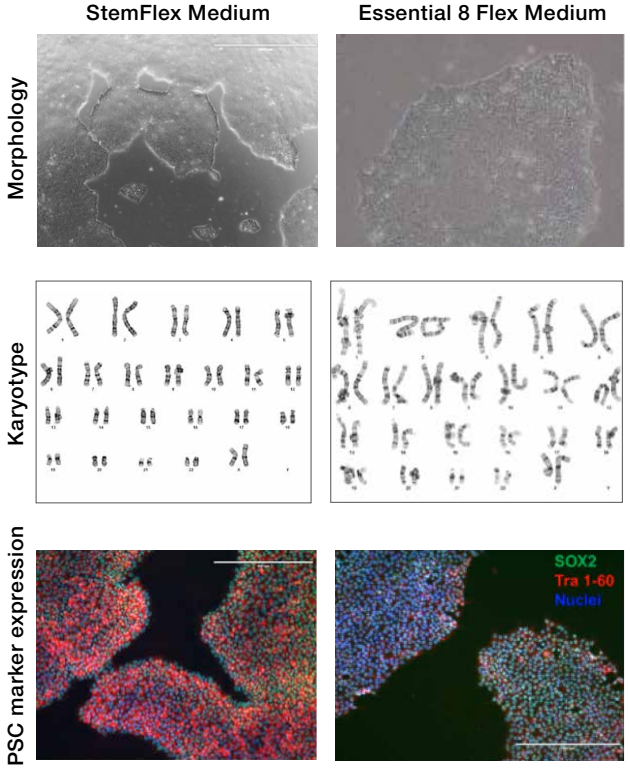


Figure 6. Long-term maintenance of pluripotency in weekend-free feeding schedules. PSCs exhibit normal morphology, karyotype, and expression of pluripotent stem cell markers following 50 passages in StemFlex Medium on a Geltrex matrix (left) and in Essential 8 Flex Medium on a Gibco™ vitronectin matrix (right).

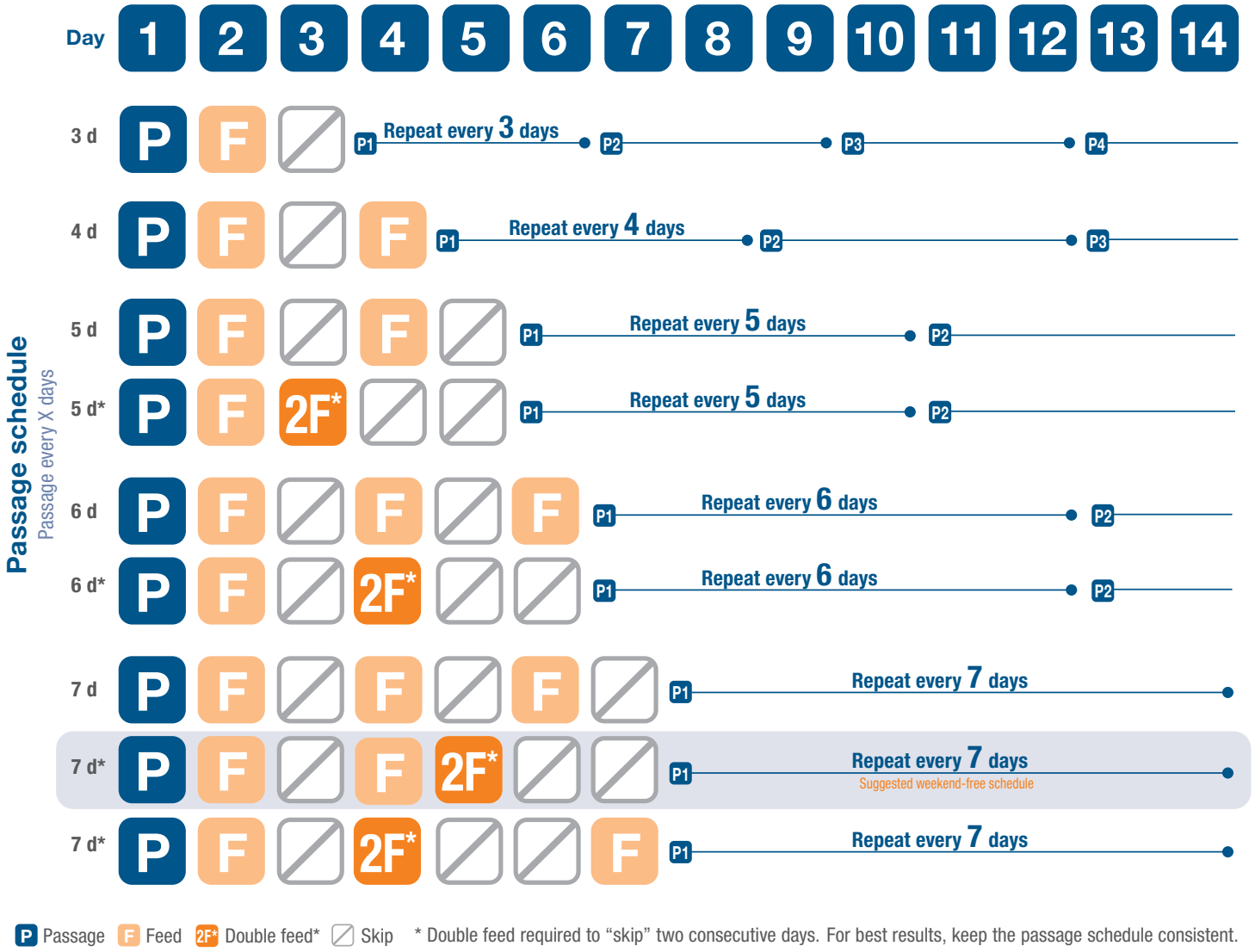


Figure 7. Alternative feed schedules for StemFlex and Essential 8 Flex media. Note: It is also possible to skip feeding the day after passaging if ROCK inhibitor is not added during passaging.

StemScale PSC Suspension Medium

Gibco™ StemScale™ PSC Suspension Medium supports the growth of PSCs in suspension through the self-assembly of spheroids. The unique formulation of StemScale medium provides superior expansion (Figure 8), while maintaining high viability and consistent spheroid formation across multiple PSC lines. Simplified medium exchange and passaging workflows offer scalability in different formats and flexibility in feeding schedule (Figure 9). Pluripotent stem cells expanded in StemScale medium maintain pluripotency and normal karyotype across multiple passages and can be differentiated to the three germ layers.

Superior expansion capability

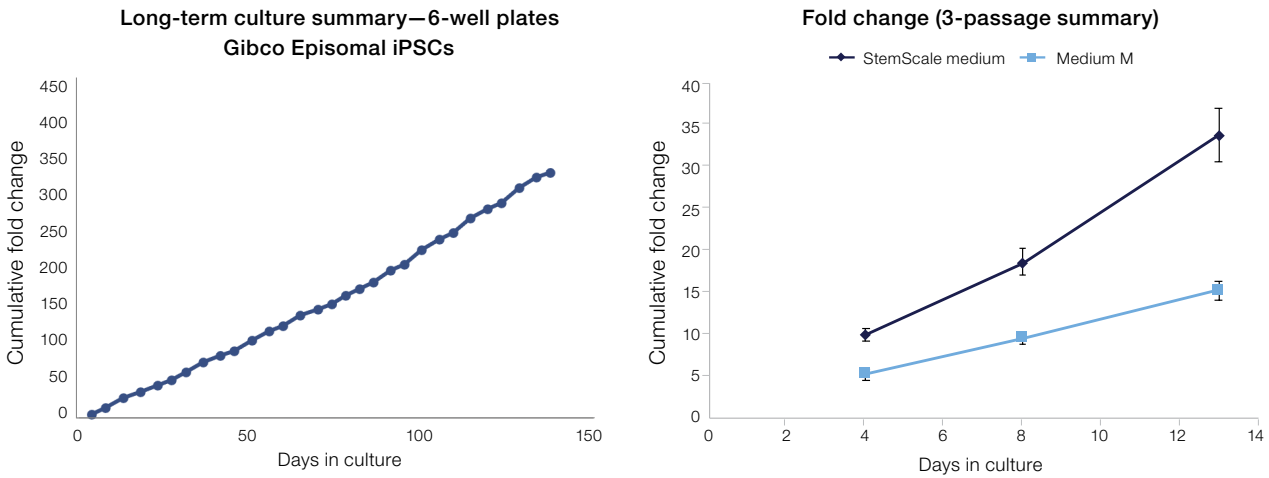


Figure 8. Scaling expansion using StemScale medium. After 30 passages and 20 weeks in culture, StemScale medium delivers greater than 10x cell expansion per passage (left plot). When compared with another commercially available medium for PSC suspension culture, StemScale medium delivers up to 3x the expansion capability across the same period (right plot).

Why choose StemScale medium?

- **Expansion capability**—delivers 5–10 fold expansion per passage and 3 times the expansion of other media
- **Workflow**—easy media changes and passaging protocols allow for scaling across multiple vessel types
- **Consistency across cell lines**—reliable and consistent spheroid formation and maintenance of pluripotency across multiple PSC lines
- **Flexible feeding schedule**—skip feeding days; passage as early as day 3
- **50% medium replacement**—prevents waste accumulation and contributes to consistent spheroid size
- **Straightforward passaging protocol**—no need for cell strainers, amenable to scale up

Find out more at **thermofisher.com/stemscale**

Superior workflow

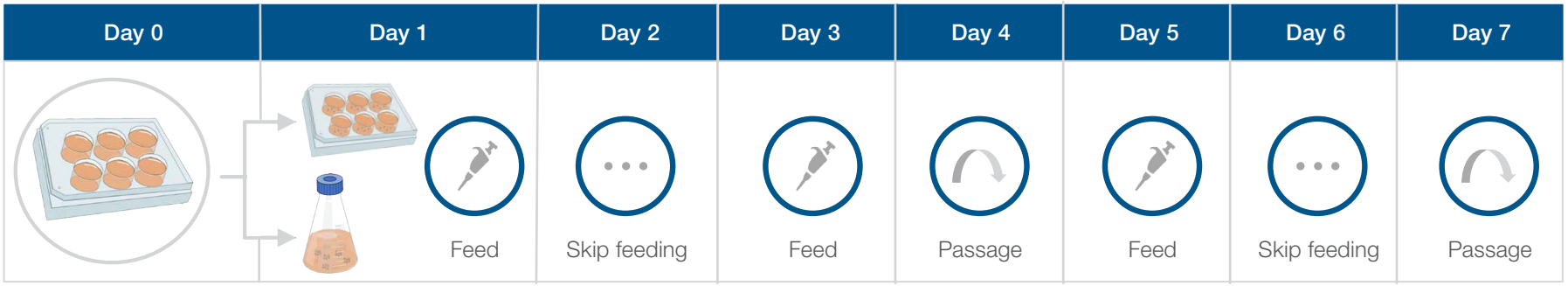


Figure 9. Simplified workflow (schematic). Easily adopt adherent cultures to suspension cultures using StemScale medium. StemScale medium enables users to skip feeding days, if desired. After initiation of cultures in StemScale medium, Gibco Episomal iPSCs are fed periodically using a 50% medium exchange, every day or every other day.

Expansion across multiple cell lines and vessel types

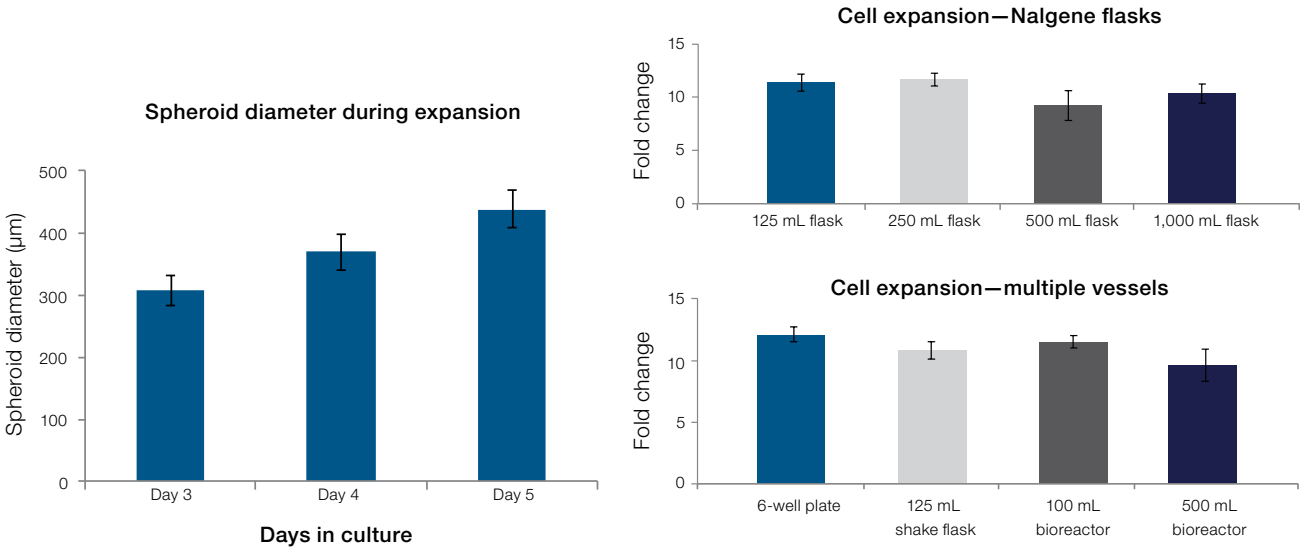


Figure 10. Suspension vs. adherent culture systems. Scale-up in suspension culture systems using StemScale PSC Suspension Medium vs. scale-up in adherent culture systems.

Essential 8 Medium

Defined and consistent stem-cell culture conditions

Essential 8 Medium is a feeder-free, xeno-free medium originally developed in the laboratory of stem cell research pioneer James Thomson. Essential 8 Medium contains only the 8 essential components needed to grow and expand PSCs, while other feeder-free stem cell media contain 20 or more components in their formulations (Table 6). These other feeder-free media may adequately grow and maintain PSCs, but they also contain many variables and commonly exhibit lot-to-lot inconsistencies. By removing highly undefined proteins and components (such as BSA) and including only the ingredients necessary for PSC culture, Essential 8 Medium helps minimize variability in culture.

Why Essential 8 Medium?

- Know what's in your media formulation and, more importantly, what's not
- No BSA or HSA
- Modular options to maximize application performance (Table 5)
- Cell therapy formulation available for clinical or translational research applications **(page 56)**

Find out more about the variations of Essential 8 Medium at **thermofisher.com/essential8media**

Table 5. Essential 8 media formulations designed for a wide variety of applications.

Application	Recommended medium	Recommended pairings
Routine PSC expansion and maintenance	Essential 8 Medium or Essential 8 Flex Medium	Vitronectin (VTN-N) Recombinant Human Protein
Superior recovery during transition to a defined, feeder-free culture system	Essential 8 Adaptation Kit	Kit includes rhLaminin-521
PSC expansion and maintenance with flexible feeding schedule	Essential 8 Flex Medium	Vitronectin (VTN-N) Recombinant Human Protein
Optimum reprogramming of somatic cells due to elimination of BSA	Essential 8 Medium or Essential 8 Flex Medium	CytoTune-iPS 2.0 Sendai Reprogramming Kit
Stressful applications in a defined media system	Essential 8 Medium or Essential 8 Flex Medium	RevitaCell Supplement rhLaminin-521
Embryoid body (EB) formation and directed differentiation	Essential 6 Medium	Nunclon Sphera Plates RevitaCell Supplement
Clinical applications	CTS Essential 8 Medium	CTS Vitronectin Matrix CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit

Table 6. Defined formulation of Essential 8 Medium compared to other published PSC media. Essential 8 Medium uses far fewer components to support PSC growth and expansion compared to mTeSR1 medium (STEMCELL Technologies). Unlike mTeSR1 medium, Essential 8 Medium does not contain bovine serum albumin (BSA), which is a source of variability.

Components	mTeSR1	Essential 8
DMEM/F-12	•	•
L-Ascorbic acid	•	•
Selenium	•	•
Transferrin	•	•
$NaHCO_3$	•	•
Insulin	•	•
FGF-2	•	•
TGFB1	•	•
Albumin (BSA)	•	
Glutathione	•	
L-Glutamine	•	
Defined lipids	•	
Thiamine	•	
Trace elements B	•	
Trace elements C	•	
β-Mercaptoethanol	•	
Pipecolic acid	•	
LiCl	•	
GABA	•	
H_2O	•	

KnockOut Serum Replacement

Feeder-dependent culture proven more reliable than FBS culture

Fetal bovine serum (FBS) is a complex mixture of components that can vary from lot to lot and can be detrimental to PSCs. More defined supplements like Gibco™ KnockOut™ Serum Replacement have more consistent compositions that reduce the number of detrimental components and retain the most critical components for PSC maintenance. KnockOut Serum Replacement is an FBS-free culture supplement designed to replace FBS in feeder-based PSC culture.

Why KnockOut Serum Replacement?

- Cited in over 4,000 publications on various PSC-related applications
- Improved maintenance of undifferentiated ESCs and iPSCs, especially when used with Gibco™ DMEM/F-12, GlutaMAX™ Supplement (Figure 11)
- Superior cryopreservation and recovery
- Directed differentiation with addition of desired growth factors; no need to transition to a different medium

Combine it with our broad offering of rigorously tested mouse embryonic fibroblasts (MEFs). Learn more at **thermofisher.com/gibcomefs**

See additional data, resources, and publications at **thermofisher.com/ksrmedia**

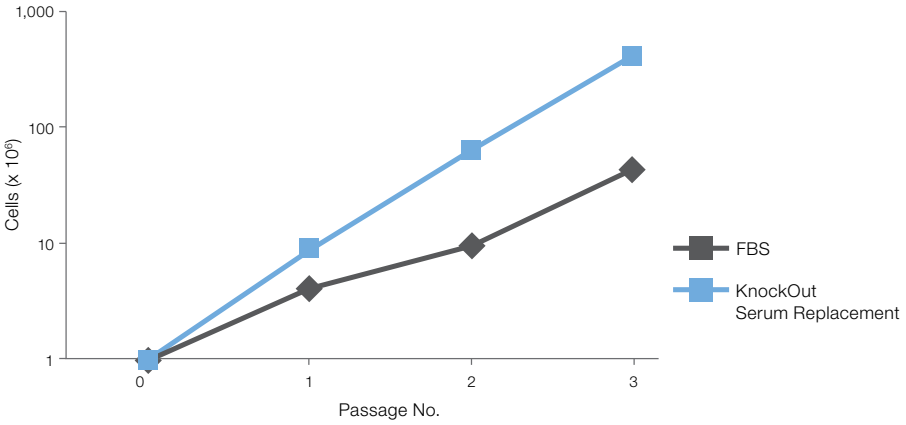


Figure 11. Human PSC growth in KnockOut Serum Replacement vs. FBS. H9 human ESCs were cultured on mouse embryonic fibroblasts (MEFs) with 20% ESC-qualified FBS or 20% KnockOut Serum Replacement. The mean viable cell numbers were plotted as growth curves for the two types of media. Proliferation of human ESCs was significantly higher in KnockOut Serum Replacement over 3 passages.

PSC cryopreservation

Cryopreservation is a critical and sometimes challenging step in your research. That's why we offer choices in Gibco™ cryopreservation technologies designed to fit your research and resource needs.

Choose your cryopreservation solution at **thermofisher.com/cryopreservation**

Table 7. Cryopreservation product overview.

Product	PSC Cryopreservation Kit	Synth-a-Freeze Cryopreservation Medium
Application	Cryopreservation medium and recovery supplement optimized for maximum viability of PSCs	For freezing and storing a variety of cell types
Tested cell types	iPSCs, ESCs, PBMCs, iPSC-derived cardiomyocytes	Human keratinocytes, PSCs, MSCs, NSCs, other primary cell types
Chemical composition	Xeno-free cryomedium; animal origin–free, chemically defined recovery supplement	Animal origin–free
Ready to use	Yes	Yes
Recovery component included	Yes, RevitaCell Supplement	No*
CTS product available	CTS PSC Cryopreservation Kit	CTS Synth-a-Freeze Cryopreservation Medium

* RevitaCell Supplement can be purchased separately and utilized in post-thaw recovery for PSCs cryopreserved in Synth-a-Freeze medium.

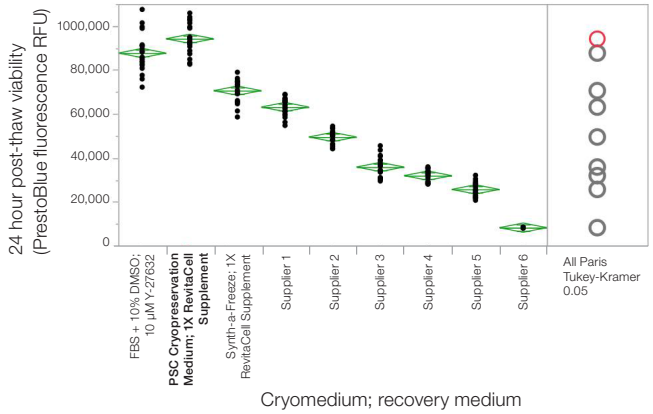


Figure 12. The Gibco™ PSC Cryopreservation Kit provides optimum 24-hour post-thaw cell survival. H9 ESCs cultured in Essential 8 Medium were cryopreserved in various cryopreservation media and subsequently recovered in Essential 8 Medium supplemented with either 10 µM Y-27632 or 1X RevitaCell Supplement. Cell viability was assessed 24 hours post-thaw using Invitrogen™ PrestoBlue™ Cell Viability Reagent, and the PSC Cryopreservation Kit was shown to provide optimal cell survival.

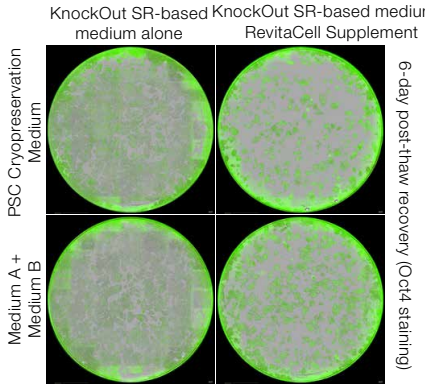


Figure 13. The PSC Cryopreservation Kit also shows utility for cryopreservation of feeder-dependent iPSCs. Feeder-dependent episomal iPSCs were cryopreserved in Gibco™ PSC Cryopreservation Medium or traditionally recommended Medium A + B. PSCs were then recovered in KnockOut Serum Replacement–based feeder-dependent medium alone or in the presence of 1X RevitaCell Supplement for the first 24 hours post-passage. Recovery was assessed 6 days post-thaw. Use of PSC Cryopreservation Medium alone affords cryopreservation capacity comparable to Medium A + B, whereas addition of the RevitaCell Supplement significantly improved cell survival.


25ml x 0.2
gibco
1X
DMEM

Cell culture plastics

To help ensure reproducible and reliable results across your stem cell workflow, we offer an extensive range of cell culture plastics in a variety of formats and surfaces.

Helpful tips for choosing your surface:

- Thermo Scientific™ Nunclon™ Sphera™ surface demonstrates superior quality for embryoid body (EB) formation of PSCs with minimal spontaneous differentiation. The resultant cells are able to differentiate into all three germ layers.
- Choose Thermo Scientific™ Nunc™ poly-D-lysine– or collagen I–coated surface, and Nunc™ Lab-Tek™ II CC²™–modified glass surface for primary cells and sensitive cells, as these surfaces imitate the growth environment of cells inside a living body, which is ideal for cells that don't grow well on the regular tissue culture surface.
- Use temperature-responsive Thermo Scientific™ Nunc™ UpCell™ surface for adherent cultures that require enzyme-free cell detachment. This surface allows harvesting of cells in single-cell suspensions or as contiguous cell sheets, and helps create 3D tissue models without artificial scaffold material.

Choose the best solutions for your stem cell workflow at **thermofisher.com/cellcultureplastics**

Support resources

- See how Nunclon Sphera plates support EB formation at **thermofisher.com/sphera**
- Download protocol to coat Thermo Scientific™ Nunc™ Lab-Tek™ chamber slides and coverglasses at **thermofisher.com/ecmcoatingprotocol**

Table 8. Cell culture plastics overview.

Stem cell workflow	Cell culture format	Cell culture surface modifications						
		Nunclon Delta for standard tissue culture, treated	Nunclon Sphera for 3D cell culture	Collagen I	Poly-D-lysine	Untreated	Nunc UpCell surface for adherent cultures that require enzyme-free cell detachment	Nunc Lab-Tek Chamber Slides with CC² glass
Maintain	Nunc flasks, dishes, and plates	•		•	•	•	•	
Reprogram	Nunc plates	•		•	•			
Culture	Nunc plates and dishes	•	•	•	•			
Engineer	Nunc plates	•	•	•	•		•	
Differentiate	Nunc plates and dishes	•	•	•	•			
Characterize	Nunc Lab-Tek and Lab-Tek II Chamber Slides and CC² glass	•						•
	Nunc Optical Bottom Plates	•				•		•
	Nunc Glass Bottom Dishes	•						


invitrogen
by Thermo Fisher Scientific
Lipofectamine® 2000
Reagent
Qty: 1.5 mL (1 mg/mL)
Lot 2160510
Exp 2021-12-31
Cat no. 11668-019
Store at: 2 °C – 8 °C
For Research Use Only. Not for use in diagnostic procedures.
Life Technologies Corp.
5781 Van Allen Way Carlsbad, CA 92008
Product of Lithuania
Read SDS
24-hour Emergency Response, Call CHEMTREC
USA & Canada: 1-800-424-9300 / +1 703-527-3887
Outside USA & Canada: +1 703-741-5970
To reorder, visit thermofisher.com
For support, visit thermofisher.com/support
Lipofectamine® CRISPRMAX
Transfection Reagent
Qty: 0.75mL
Lot 2185373
Exp 2021-02-18
Cat no. CMAX00008
Store at: 2 °C – 8 °C
Lipofectamine® RNAiMAX
Reagent
Qty: 1.5 mL
Lot 2173184
Exp 2022-02-07
Cat no. 13778-150
Store at: 2 °C – 8 °C
Cas9 Plus™ Reagent
Qty: 1250 µL
Research Use Only
Carlsbad, CA

Transfection

Transfection is the process by which nucleic acids are introduced into eukaryotic cells. Techniques vary widely and include lipid nanoparticle–mediated transfection and physical methods such as electroporation. Invitrogen™ Lipofectamine™ transfection reagents are among the most trusted and cited in the scientific literature due to their superior transfection performance and broad cell spectrum.

Choose the solution that's right for you at **thermofisher.com/transfection**

Support resources

- View transfection protocols at **thermofisher.com/transfectionprotocols**
- Explore transfection resources at **thermofisher.com/transfectionbasics**

Table 9. Transfection selection guide for stem cells.*

Transfection method	Recommended payloads				Transfection efficiency by cell type			
	DNA	mRNA	Ribonucleoprotein (Cas9 protein)	Co-delivery	iPSC	ESC	NSC	MSC
Lipofectamine Stem reagent	✓	✓	✓	✓	■■■■	■■■■	■■■■	■■□□
Lipofectamine 3000 reagent	✓			✓	■■■□	■■■□	■■□□	NA
Lipofectamine MessengerMAX reagent		✓			■■■□	■■■□	■■□□	■■□□
Neon Transfection System	✓	✓	✓	✓	■■■■	■■■■	■■■□	■■■■
Lipofectamine CRISPRMAX reagent	✓		✓		■■■□	Not tested	Not tested	Not tested

*Recommended payloads by transfection method, and transfection efficiency by cell type, are shown. A higher number of blue blocks represent higher efficiency.

Need help transfecting your cells?
Thermo Fisher Scientific employs a dedicated team of stem cell scientists to help you achieve your project goals. See the section starting on **page 58** for all of our stem cell services.

Lipofectamine Stem Transfection Reagent

Achieve the optimal balance of high efficiency and low toxicity with this breakthrough stem cell transfection reagent

Invitrogen™ Lipofectamine™ Stem Transfection Reagent is our premier transfection reagent for stem cells. It was developed to achieve maximum efficiency without toxicity across stem cell types, payloads, and media. It can deliver large constructs and is highly effective for gene editing, gene expression, and directed differentiation.

Why Lipofectamine Stem reagent?

- **Superior efficiency**—achieve up to 80% transfection efficiency in PSCs and NSCs and up to 45% in MSCs
- **Versatility**—co-delivers DNA (up to 11 kb), RNA, and Cas9 protein complexes; continues cell proliferation without inducing differentiation (Figure 14)
- **Flexibility**—transfects adherent and suspension cells, offering a simple alternative to electroporation

Find out more at **thermofisher.com/lipofectaminestem**

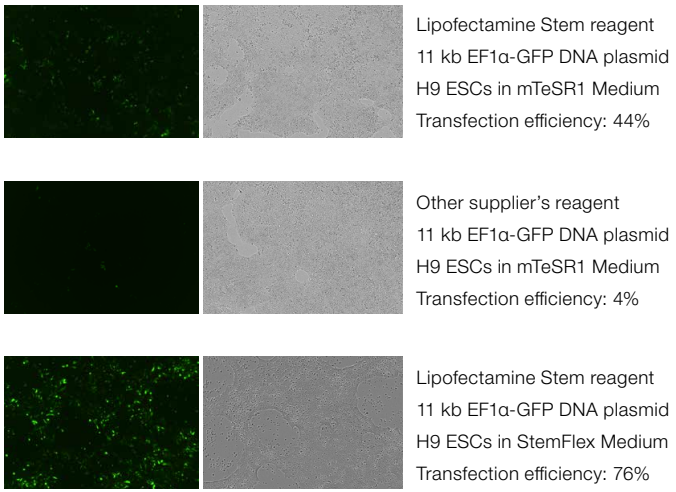


Figure 14. Delivery of a large DNA construct, with significantly higher transfection efficiency than that achieved using a leading supplier's reagent. H9 ESCs were transfected with a large 11 kb DNA plasmid using **(A)** Lipofectamine Stem reagent or **(B)** a leading supplier's transfection reagent, and observed 24 hours posttransfection. **(C)** By optimizing culture conditions for transfection, efficiency was nearly doubled.

Lipofectamine 3000 Transfection Reagent

Achieve over 60% transfection efficiency in stem cells

Invitrogen™ Lipofectamine™ 3000 Transfection Reagent with P3000™ Reagent was developed as a customizable DNA delivery reagent that achieves efficient nucleic acid delivery across hard-to-transfect cell types including stem cells. It minimizes stress on cells when compared to electroporation, simplifies the reprogramming workflow, and enables advanced gene-editing technologies.

Why Lipofectamine 3000 reagent?

- Up to 10x higher efficiency for the broadest spectrum of difficult-to-transfect cells (Figure 15)
- Reduced toxicity by customizing the amount of reagent best suited for your cell type
- Highly efficient reprogramming of somatic cells without the need for electroporation

Learn more at **thermofisher.com/3000**

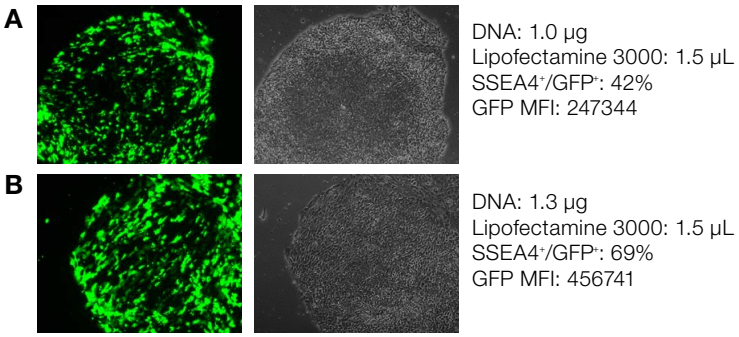


Figure 15. Transfection of stem cells. (A) H9 ESCs or **(B)** iPSCs were transfected using Lipofectamine 3000 reagent. Cells were stained for pluripotency with an SSEA4 antibody, visualized by fluorescence microscopy, and processed using flow cytometry to determine transfection efficiency and SSEA4⁺ cells.

Lipofectamine MessengerMAX Transfection Reagent

High transfection efficiency in stem cells, primary cells, and neurons

Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent delivers over 60% transfection efficiency in stem cells, primary cells, and neurons.

Why Lipofectamine MessengerMAX reagent?

- Faster protein expression with no risk of genomic integration
- Up to 10x higher cleavage efficiency using Invitrogen™ GeneArt™ CRISPR Nuclease mRNA
- Direct delivery to cytoplasm—great for slow-dividing cells

Learn more about superior mRNA transfection efficiency at **thermofisher.com/messengermax**

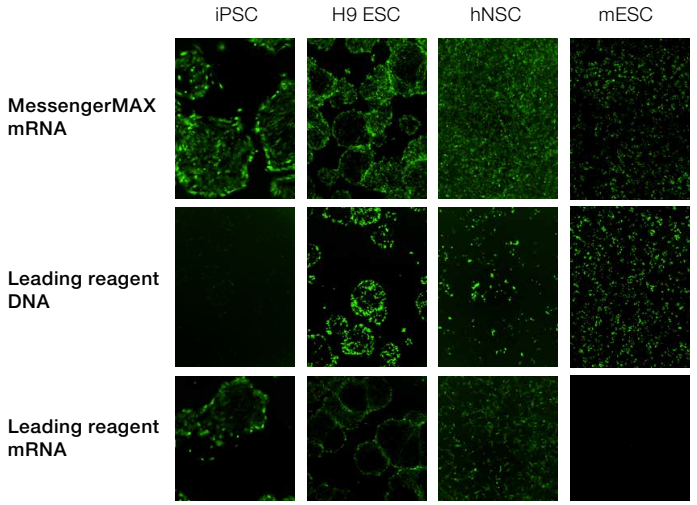


Figure 16. Lipofectamine MessengerMAX reagent outperforms leading DNA delivery reagent and leading mRNA delivery reagent in various stem cells (Gibco iPSCs, H9 ESCs, mESCs, and hNSCs). Lipofectamine MessengerMAX and the leading mRNA delivery reagent were used to deliver green fluorescent protein (GFP) mRNA (250 ng/well) in a 48-well format. The leading DNA delivery reagent was used to deliver GFP DNA (250 ng/well), and GFP was analyzed 24 hours posttransfection.

Neon Transfection System

Simple, customizable, and gentle electroporation instrument that delivers high transfection and cleavage efficiency

The Invitrogen™ Neon™ Transfection System is an electroporation device for highly efficient transfection and gene editing of primary cells, stem cells, and hard-to-transfect cells.

Find out more about superior electroporation performance at **thermofisher.com/neon**

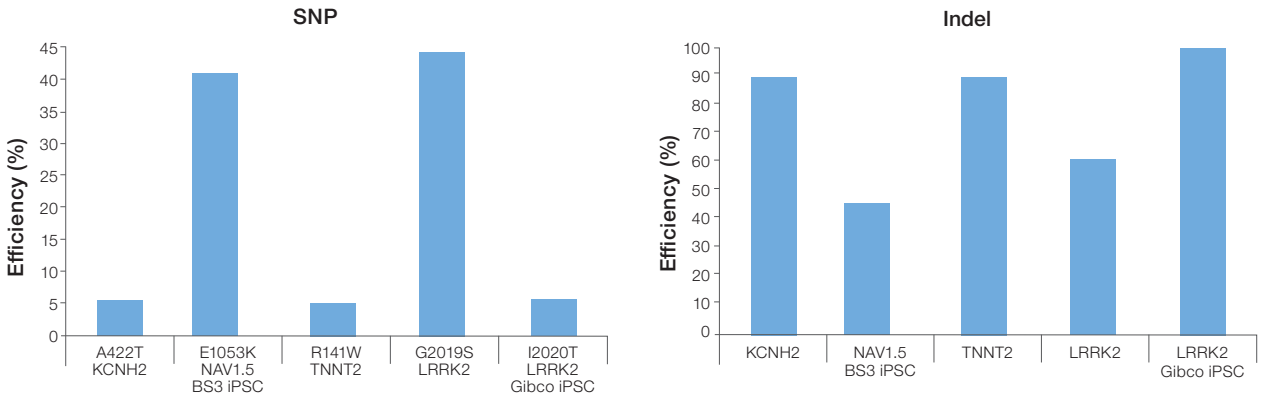


Figure 17. Delivery of Cas9 protein and gRNAs via Neon electroporation allows for efficient genome editing in hiPSC lines across multiple targets. Both NHEJ- and HDR-mediated genome editing can be achieved efficiently, although they target dependently. SNP = single nucleotide polymorphism

Why Neon Transfection System?

- **Superior results**—up to 90% transfection efficiency and 85% cleavage efficiency in stem cells
- **Gentle**—the novel pipette tip chamber requires fewer cells per experiment, generates a more uniform electric field than cuvettes, is gentler on cells, and enables higher efficiency
- **Customizable**—preprogrammed 24-well optimization protocols and an open platform for additional protocols enable more gentle transfection and higher viability than other electroporation instruments
- **Compatible**—use with StemFlex Medium during genome editing via electroporation

Lipofectamine CRISPRMAX Transfection Reagent

Demonstrated cleavage efficiency in iPSCs

Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent increases the likelihood of successful cleavage and recombination with efficient delivery of Invitrogen™ TrueCut™ Cas9 Protein v2 and Invitrogen™ TrueGuide™ Synthetic gRNA. This ultimately maximizes the efficiency of performing genetic modifications, even in hard-to-transfect cells such as human iPSCs.

Why Lipofectamine CRISPRMAX reagent?

- **Demonstrated cleavage efficiency**—over 50% cleavage efficiency in iPSCs
- **Support of multiple cell lines**—tested in more than 20 cell types including iPSCs, mESCs, N2A, CHO, A549, HCT116, HeLa, HEK293, and several others
- **Low cell toxicity**—fewer cells needed to initiate your experiment
- **Cost savings**—lower cost per reaction and lower initial investment
- **Easy scalability**—an ideal delivery solution for high-throughput experiments

Find out more at **thermofisher.com/crisprmax**

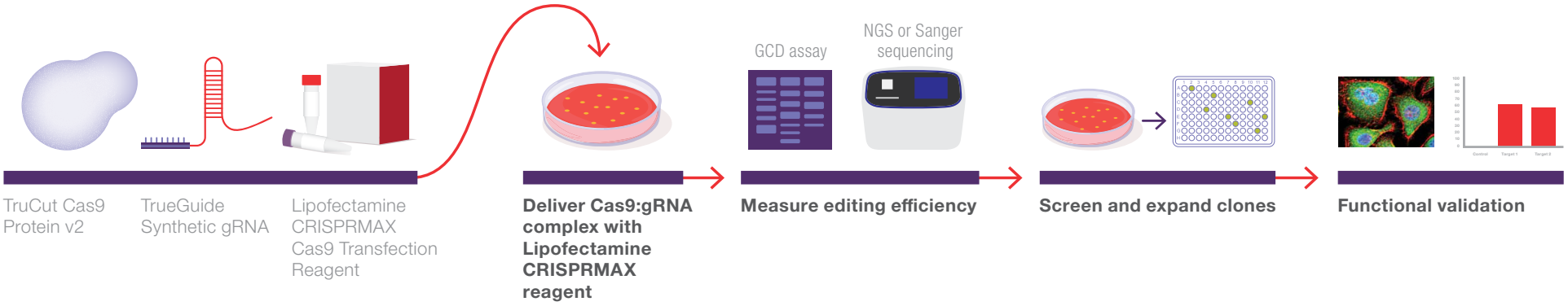
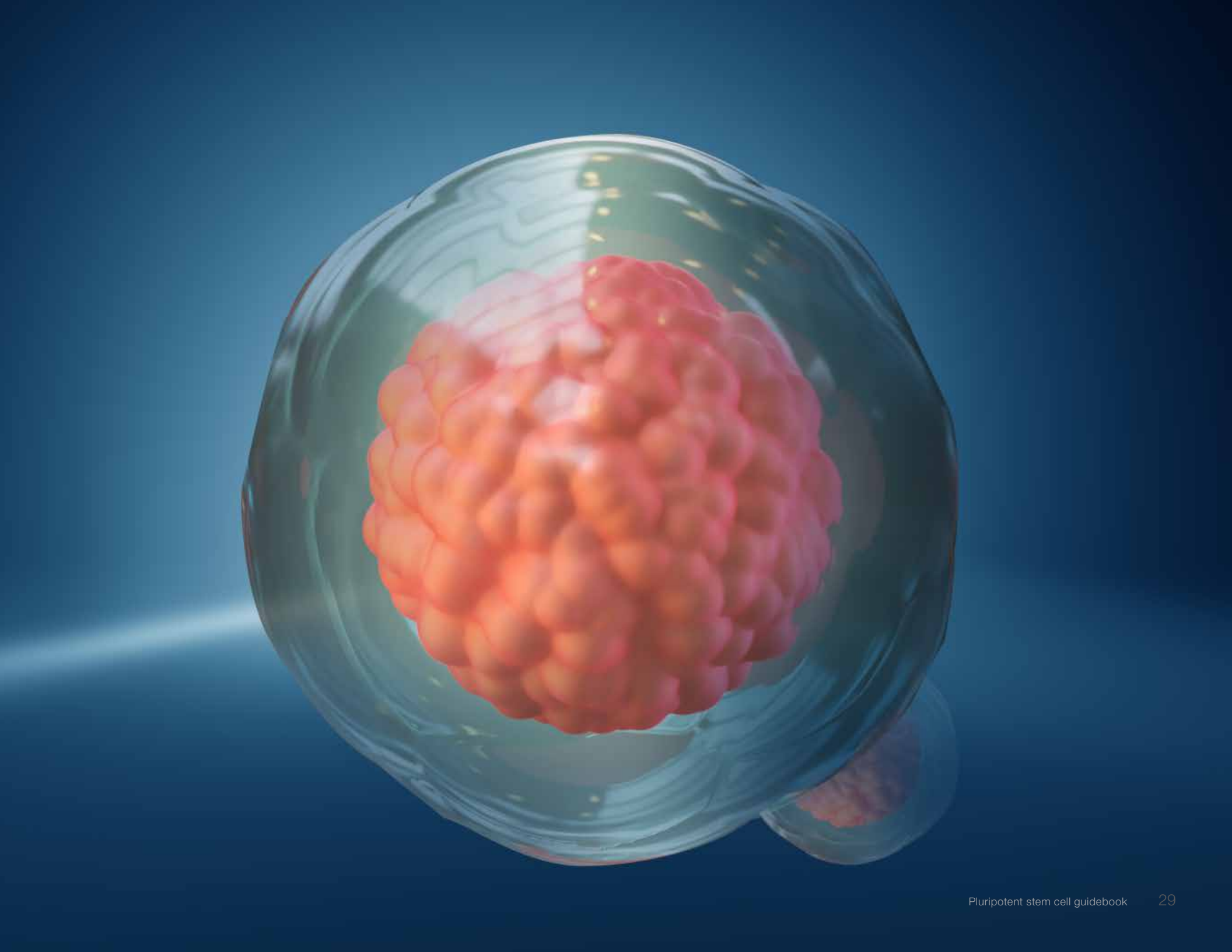


Figure 18. Genome editing workflow featuring Lipofectamine CRISPRMAX delivery technology.



Genome editing

Genome editing technologies, such as CRISPR-Cas9 and transcription activator-like (TAL) effector nucleases (TALENs), provide researchers precise and efficient methods for manipulating genomic DNA sequences. Whether you are seeking to knock out a specific gene or introduce (or correct) a specific mutation, the combination of genome editing tools and stem cells allows you to build organ- and disease-specific models to drive understanding of how individual genes and mutations influence disease development and progression. Our collection of optimized genome editing tools are designed to work together to minimize the trial-and-error phase and help you develop models faster and with less effort.

Find out more about our genome editing products and services at **thermofisher.com/genomeedit**

Support resources

- New to genome editing? Access our 24/7 learning center at **thermofisher.com/genomeedit101**
- Download the Genome Editing Resource Guide at **thermofisher.com/crisprguide**
- Need help designing your experiments? Try our free, online design tool: TrueDesign Genome Editor at **thermofisher.com/truedesign**

Table 10. Genome editing product selection guide.

	Single-gene analysis		High-throughput screening	
End goal	Permanent gene knockout or knock-in		Transient gene knockdown	Permanent gene knockout
Technology	**CRISPR-Cas9**	**TALEN**	**RNAi**	**CRISPR-Cas9**
Benefits	• Superior cleavage efficiency • Simple design and assembly process • Multiplexing capable	• Flexible; no sequence restriction or protospacer adjacent motif (PAM) requirement; ideal for knock-in • Includes the rights under foundational TAL IP	• Ultimate flexibility in technology and gene targets • High-potency gene silencing • Minimal off-target effects	• Superior cleavage efficiency • No cell-specific promoter constraint • No random integration concern
Design requirement	PAM site (NGG)	Completely flexible, no design restrictions	Genome-wide predesigned reagents are available	PAM site (NGG)
Ideal products for PSC	TrueCut Cas9 Protein v2, TrueCut HiFi Cas9 Protein, TrueGuide Synthetic gRNAs, TrueTag Donor DNA Kits	FlexCut TALEN mRNA	*Silencer* Select siRNA libraries	LentiArray CRISPR libraries or Custom LentiPool CRISPR libraries
Format	NA	NA	Arrayed libraries	Arrayed or pooled libraries

TrueCut Cas9 proteins

Next-generation Cas9 proteins designed to deliver maximum editing efficiency

Invitrogen™ TrueCut™ Cas9 proteins are designed to deliver consistently high editing efficiency across a range of gene targets and cell types. We offer two performance-leading Cas9 proteins to better meet your genome editing goals—Invitrogen™ TrueCut™ Cas9 Protein v2*, for most common research applications, and Invitrogen™ TrueCut™ HiFi Cas9 Protein, for applications that are more sensitive to off-target effects. Both high-quality Cas9 proteins are manufactured under ISO 13485 quality standards.

* Available in 2.5 mg and 5 mg packs for larger-scale cell engineering.

Choose the right Cas9 protein for your specific genome editing needs at **thermofisher.com/cas9**

Why TrueCut Cas9 Protein v2?

- **Exceptional editing efficiency**—consistently high on-target editing efficiency in all tested cell lines, including standard, primary, stem, and immune (>90% editing in T cells)
- **Superior performance**—up to 2x higher editing efficiency in difficult targets than the competition
- **Confidence in results**—Validated protocols for a large number of cell types help you achieve success faster. Access these protocols at **thermofisher.com/crisprprotocols**

Why TrueCut HiFi Cas9 Protein?

- **Improved specificity**—significantly reduce off-target effects in a broad range of cell types including primary, stem, and immune cells
- **High editing efficiency**—preserved high on-target editing efficiency relative to the TrueCut Cas9 Protein v2 (wild type)

Need help engineering your cells?

Thermo Fisher Scientific employs a dedicated team of stem cell scientists to help you achieve your project goals. See the section starting on **page 58** for all of our stem cell services.

CRISPR-Cas9 editing tools

Maximum flexibility of high-quality Cas9 nuclease and CRISPR gRNAs

To successfully perform CRISPR-Cas9–mediated genome editing of mouse pluripotent stem cells (mPSCs), many factors need to be considered, such as choice of growth media, genome editing tools, and delivery methods. For editing human PSCs, we recommend StemFlex Medium, which is optimized to support single-cell applications. For mPSCs, we offer a protocol using KnockOut Serum Replacement. Below is a guide for various formats of Cas9 nuclease and CRISPR gRNA as well as the recommended transfection methods to use with each one.

Custom engineering tools, designer cell lines, libraries, and services

Even with advanced genome editing tools, it can take time to isolate and validate edited clones. To help ensure you have what you need to get your results faster, we now offer custom design and cell engineering services, including Cas9-stable cell lines and Cas9 iPSCs. From start to finish, accelerate your discovery by partnering with us.

See custom services and cell lines at **thermofisher.com/celllineservice**

Find out more or place your order at **thermofisher.com/crispr**

Table 11. Invitrogen™ CRISPR-Cas9 product formats.

	Cas9 nuclease				CRISPR gRNA			
Formats available	Cas9 protein	Cas9 mRNA	Cas9 lentivirus	Cas9 plasmid	Predesigned, ready-to-use synthetic gRNA	Custom, ready-to-use synthetic gRNA	*In vitro* transcribed gRNA	Ready-to-use lentivirus gRNA
Editing product	**Award-winning*** TrueCut Cas9 Protein v2 TrueCut HiFi Cas9 Protein	GeneArt CRISPR Nuclease mRNA	LentiArray Cas9 Lentivirus	GeneArt CRISPR Nuclease Vector	TrueGuide Synthetic sgRNA, predesigned	TrueGuide Synthetic sgRNA, custom	Precision gRNA Synthesis Kit	LentiArray lentiviral sgRNA
Recommended delivery product	Lipofectamine Stem reagent or Neon Transfection System	Lipofectamine Stem reagent or Neon Transfection System	Lentiviral transduction	Lipofectamine Stem reagent or Neon Transfection System	Delivery method determined by Cas9 nuclease format and cell type			Lentiviral transduction

* Awarded Top 10 Innovations in 2017 by *The Scientist* magazine.

Did you know that TrueGuide Synthetic sgRNA products can be designed and ordered via Invitrogen™ TrueDesign™ Genome Editor, the free design software for CRISPR-Cas9 editing?

Learn more at **thermofisher.com/truedesign**

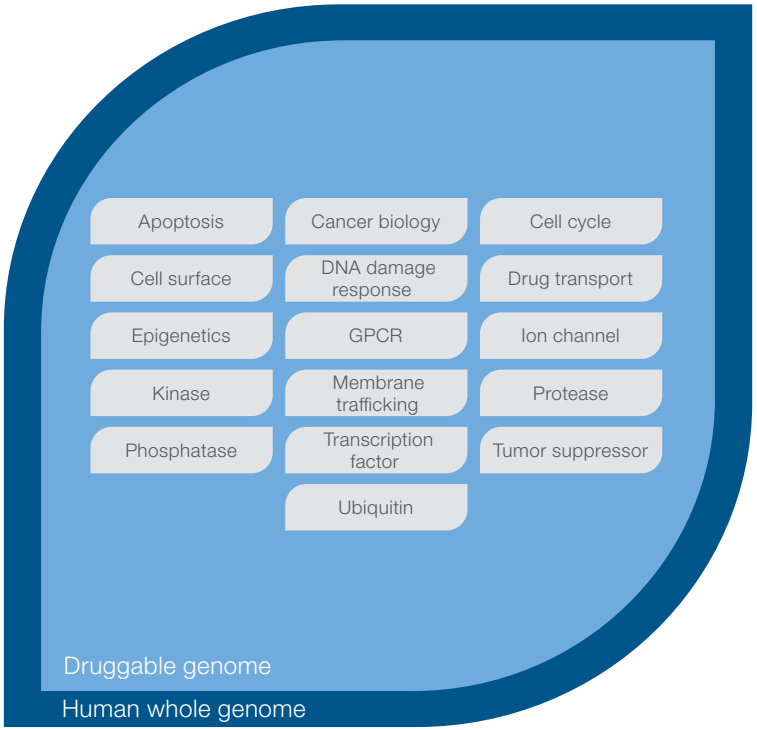
Award-winning CRISPR libraries

Bring the power of CRISPR-Cas9 technology to high-throughput screening

The CRISPR-Cas9 system is the premier technology for knocking out gene expression and is becoming a popular tool for high-throughput screening. The CRISPR-Cas9 system provides an efficient method for specific, complete, and permanent gene knockout. We are applying the power of the CRISPR-Cas9 system to high-throughput screening applications with the award-winning Invitrogen™ LentiArray™ libraries offered by Thermo Fisher Scientific. These arrayed CRISPR libraries, awarded Top 10 Innovations in 2017 by *The Scientist* magazine, are designed to provide you with flexible systems that can be adapted to your needs and help drive new discoveries.

Explore the complete CRISPR library portfolio and find out how you can apply the power of CRISPR-Cas9 technology to your screening efforts. We offer a LentiArray CRISPR Human Whole Genome Library, ideal for whole genome surveys to identify novel targets in biological pathways and disease development, and the LentiArray CRISPR Human Druggable Genome Library, ideal for identifying potential therapeutic targets involved in the development and progress of disease.

Find out more or request a quote at
thermofisher.com/crisprlibraries



Human episomal Cas9 iPSC line

To create a more robust platform for iPSC genome editing, the scientists at Thermo Fisher Scientific stably integrated the Cas9 protein into the Gibco™ Human Episomal iPSC Line.

When used in combination with CRISPR technologies, this cell line offers:

- **Performance**—up to 85% cleavage achieved
- **Quality**—extensive characterization to confirm karyotype, pluripotency potential, and genome editing efficiency
- **Flexibility**—ability to differentiate into your desired terminal cell type following editing

To gain access to the human episomal Cas9 iPSC line, submit an inquiry at
thermofisher.com/askdiscovery

Workflow for genome editing in stem cells

Gene engineering or genome editing involves changing an organism's DNA through sequence disruption, replacement, or addition. While approaches for genetic manipulation of mouse ESCs have been widely used for decades in the generation of transgenic mouse models, recent advances in genome editing technologies enable this tool to be readily applied to hPSCs.

As researchers have begun to explore workflows for gene editing in hPSCs, some common challenges cited include gene editing efficiency, and cell viability and proliferation following the manipulations. The recommended products and workflow below alleviate these common challenges, standardizing the gene editing workflow and allowing researchers to focus on their research.

Electroporation-based workflow

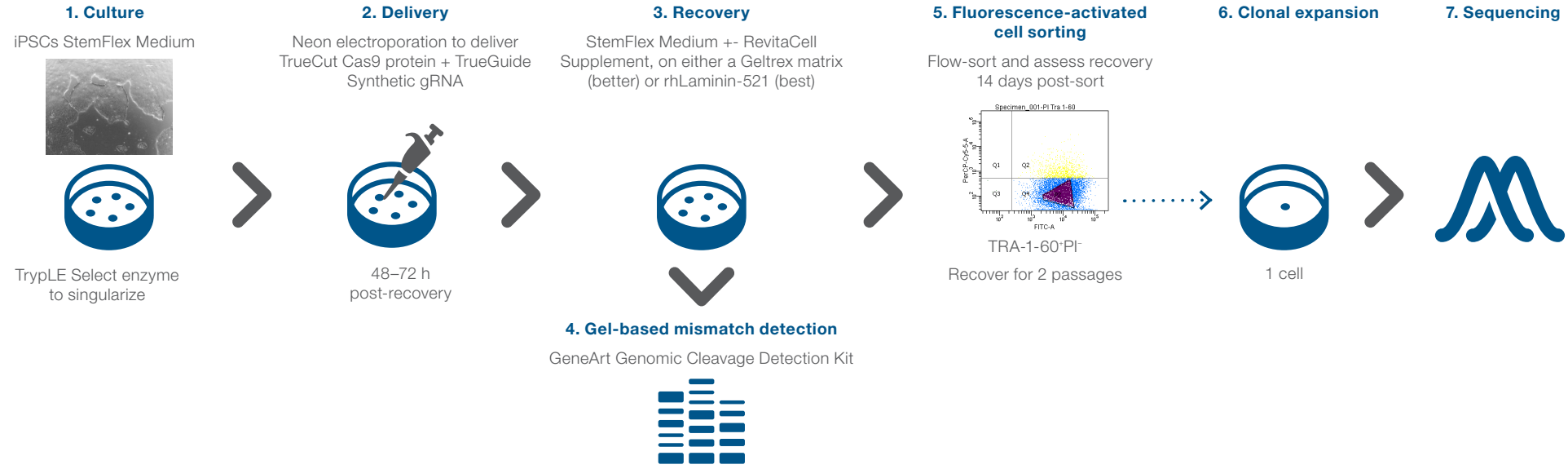


Figure 19. Standard gene editing workflow using CRISPR-Cas9 technology. Following the expansion of hPSCs with the StemFlex Medium and a Geltrex matrix system, cells are singularized and electroporated using the Neon system to introduce precomplexed Cas9 protein and control gRNA. Cells recover in StemFlex Medium in the presence or absence of RevitaCell Supplement on either Geltrex substrate or rhLaminin-521. Following 48–72 hours of recovery, cleavage efficiency is assessed using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit. Pending successful cleavage, cells recover and expand for 2 passages prior to clonal expansion. During this time, viable PSCs are flow-sorted based on expression of TRA-1-60 and the absence of PI expression. Subsequently, cells are plated at either 1 cell, 3 cells, or 5 cells per well of a 96-well plate, replacing spent medium every 3 days. Following 14 days of recovery, successful clonal expansion is determined, followed by determination of successful gene editing of clonally established lines through sequencing.

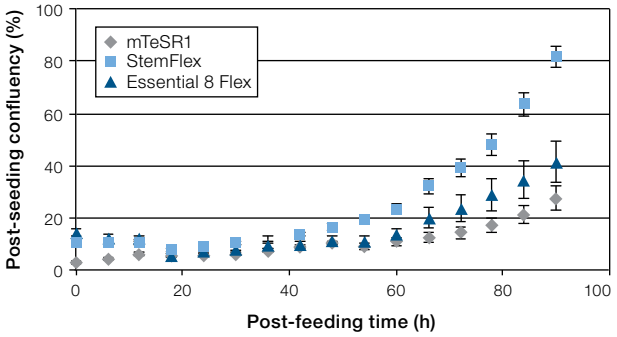


Figure 20. Recovery after singularization and electroporation with Cas9 protein and guide RNA. Cells were seeded at 100,000 viable cells/well of a 24-well plate and allowed to recover in different media. Data shown were generated with cells recovered on a Geltrex matrix.

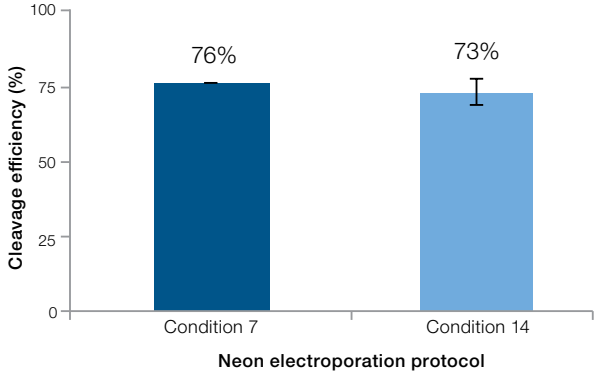


Figure 21. Cleavage efficiency of cultures grown in StemFlex Medium ~72 hours after electroporation with Cas9–gRNA complexes targeting the *HPRT* gene. Condition 7 is 1,200 V, 30 ms pulse width, and 1 pulse number; whereas condition 14 is 1,200 V, 20 ms pulse width, and 2 pulse number.

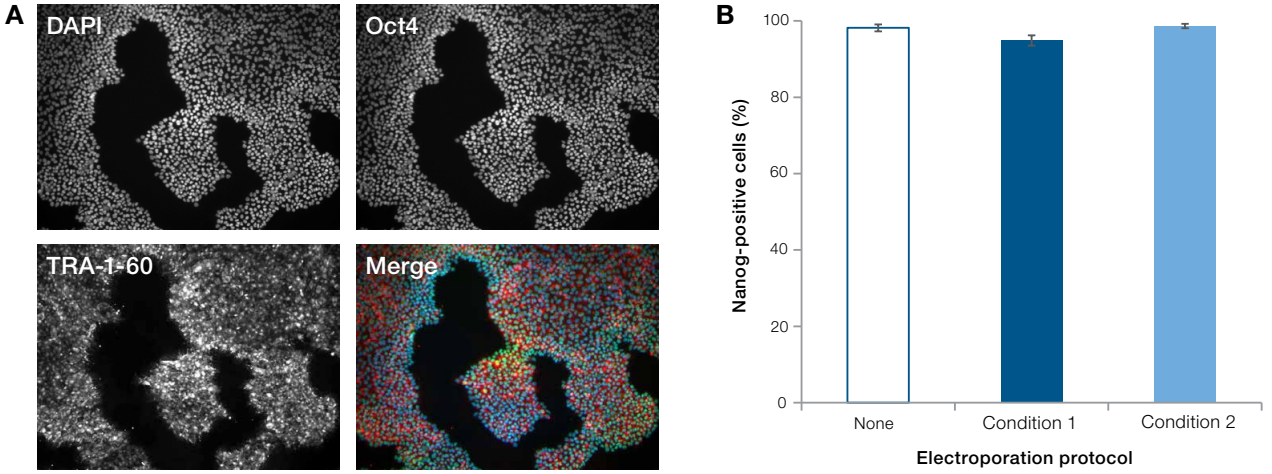


Figure 22. Maintenance of pluripotency of iPSCs cultured in StemFlex Medium after electroporation and recovery. Cultures transfected with Cas9–gRNA complexes targeting the *HPRT* gene were assessed by **(A)** qualitative immunocytochemistry of Oct4 and TRA-1-60 expression and **(B)** quantitative assessment of Nanog expression via flow cytometric analysis.

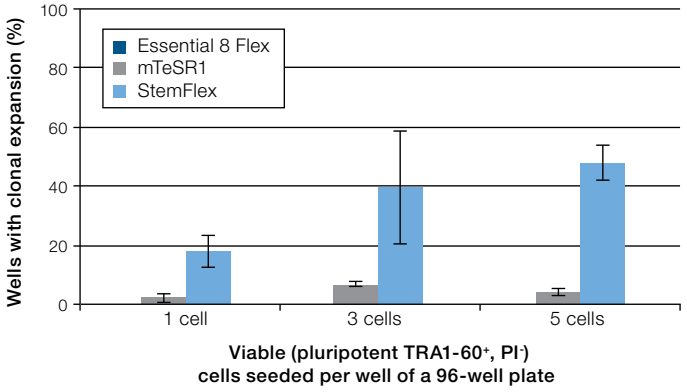


Figure 23. Comparison of cell recovery following flow sorting. Cells were evaluated in the three different media without the need for a ROCK inhibitor, and plated at 1, 3, or 5 cells per well on rhLaminin-521. These data demonstrate that StemFlex Medium is the only system that enables significant clonal expansion when a single cell is plated per well, and the medium does this even in the absence of the RevitaCell Supplement (or a ROCK inhibitor). Note that the addition of RevitaCell Supplement further boosts cell recovery.

Lipid-based transfection workflow

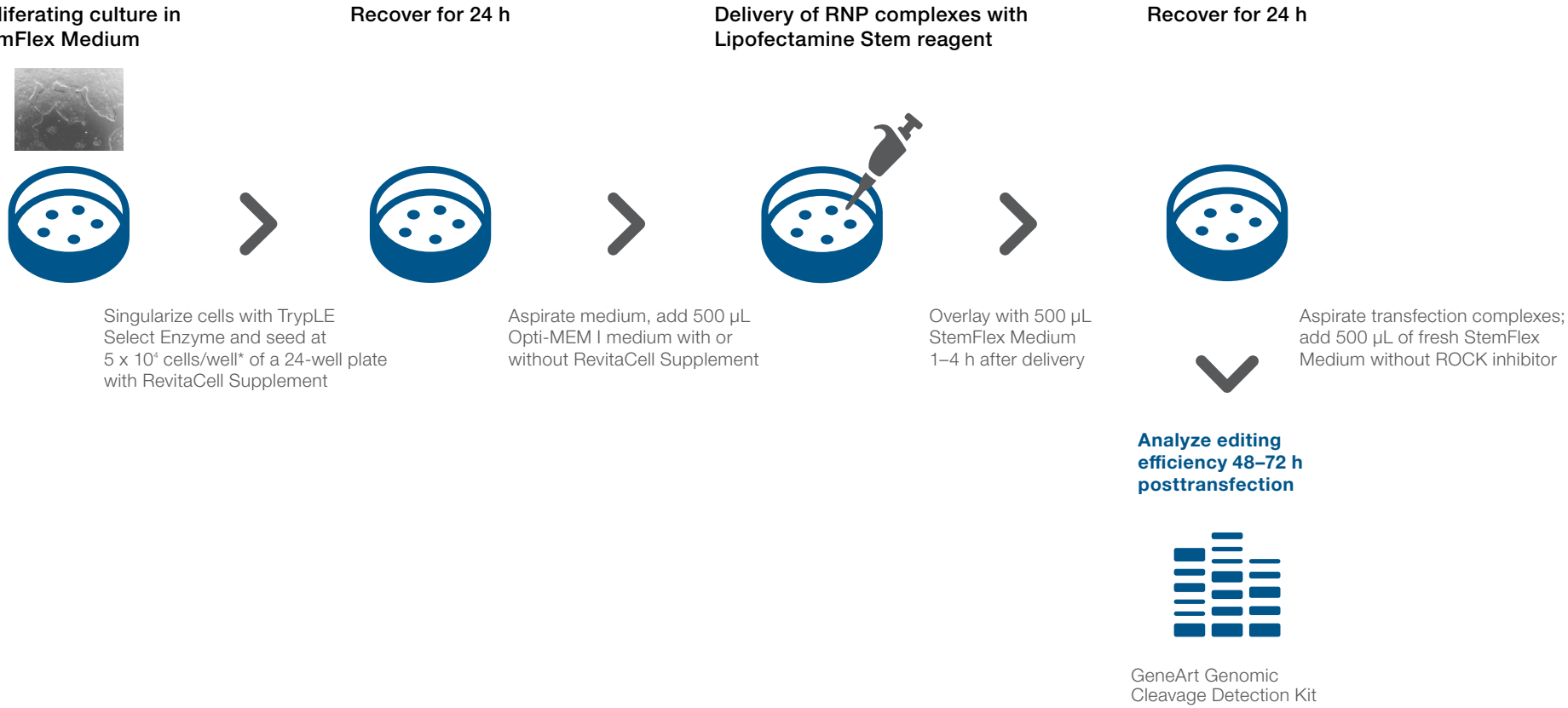


Figure 24. Transfection workflow for delivery of Cas9–gRNA complexes to PSCs cultured in StemFlex Medium using Lipofectamine Stem reagent.

* Or cell line–specific seeding density to attain 30–60% confluency 24 hours post-passaging.

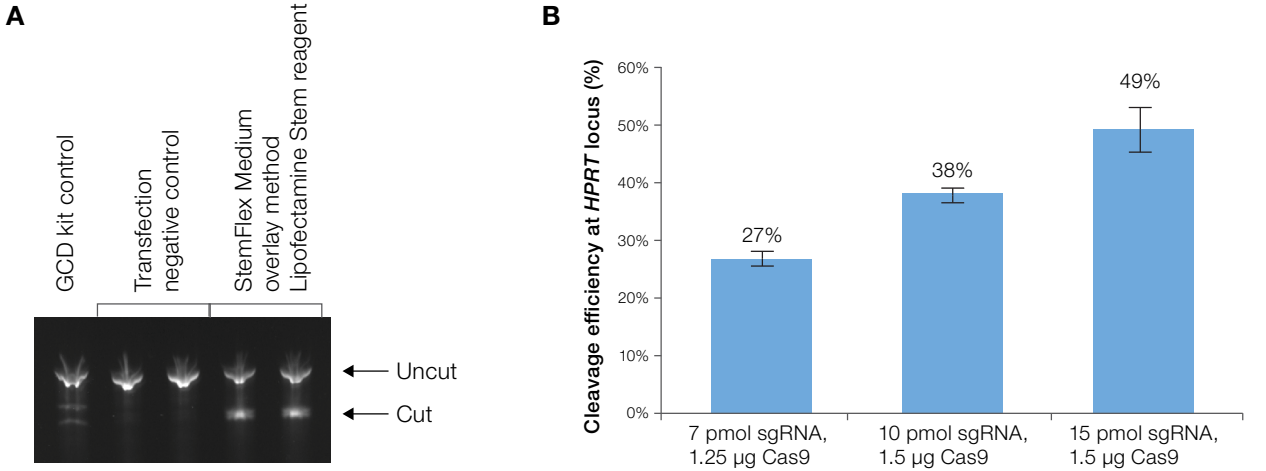


Figure 25. Assessment of indel formation following delivery of Cas9 RNP complex via Lipofectamine Stem reagent. PSCs adapted to StemFlex Medium were single cell–passaged using Gibco™ TrypLE™ Select enzyme and seeded in StemFlex Medium with 1X RevitaCell Supplement at 50,000 viable cells/cm² into 24-well Thermo Scientific™ Nunc™ Cell-Culture Treated Multidishes. Approximately 24 hours post-passaging, PSCs were transfected using the overlay method with 2 µL of Lipofectamine Stem Transfection Reagent, 1.5 µg of TrueCut Cas9 Protein v2, and 10 pmol Invitrogen™ TrueGuide™ sgRNA Positive Control, HPRT, per reaction. Following 4 hours of treatment, complexes were overlaid with 500 µL of StemFlex Medium, and subsequently the medium was replenished daily posttransfection. At 96 hours posttransfection, cells were harvested and successful indel formation was assessed using the GeneArt Genomic Cleavage Detection Kit. **(A)** H9 ESC cultures were shown to have successful indel formation at the HPRT locus with 43.9 ± 0.11% cleavage efficiency. **(B)** The Gibco Human Episomal iPSC Line, adapted to StemFlex Medium, was transfected using the overlay method as described above, with the exception that a range of quantities of Cas9/sgRNA were utilized. These data show that the formation of indels is titratable. For most loci, we recommend 1.5 µg of TrueCut Cas9 Protein v2 to complex with 10–20 pmol of TrueGuide sgRNA.

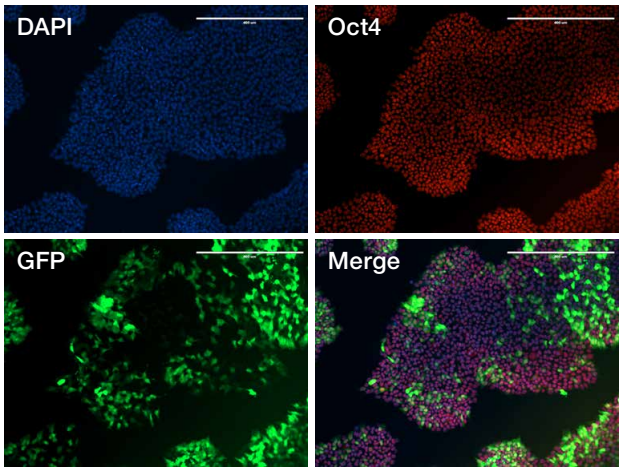


Figure 26. Representative images of transfection efficiency and maintenance of pluripotency. The Gibco Human Episomal iPSC Line, adapted to StemFlex Medium, was single cell–passaged using TrypLE Select enzyme and seeded in StemFlex Medium with 1X RevitaCell Supplement at 50,000 viable cells/cm² into 24-well Nunc Cell-Culture Treated Multidishes. Approximately 24 hours post-passaging, PSCs were transfected using the overlay method with 2 µL of Lipofectamine Stem Transfection Reagent, 1.5 µg of TrueCut Cas9 Protein v2, and 10 pmol TrueGuide sgRNA Positive Control, HPRT, per reaction. As a proxy for the transfection efficiency, 150 ng of GFP mRNA was co-delivered. Following 4 hours of treatment, complexes were overlaid with 500 µL of StemFlex Medium, and subsequently the medium was replenished daily posttransfection. At 96 hours posttransfection, cells were fixed and stained for Oct4, an intracellular marker of pluripotency. High maintenance of pluripotency was observed posttransfection using the Lipofectamine Stem reagent.


gibco
Neurobasal® Medium
gibco
Cardiomyocyte Differentiation Medium A
gibco
Cardiomyocyte Differentiation Medium B
gibco
Cardiomyocyte Maintenance Medium
gibco
PSC Definitive Endoderm Induction Medium A
gibco
PSC Definitive Endoderm Induction Medium B
gibco
Floor Plate Cell Expansion Base

Differentiation

Whether for basic research, drug discovery, or future therapeutic applications, stem cell differentiation requires standardized culture methods to ensure reproducible and reliable results. Gibco media, supplements, and substrates provide you with an easy-to-use, flexible set of tools for targeted differentiation to your desired cell lineage. Our differentiation portfolio helps simplify your workflow and provides you with more control—enabling faster, more efficient systems.

View the complete differentiation portfolio at **thermofisher.com/differentiation**

Support resources

- View differentiation protocols at **thermofisher.com/pscprotocols**
- Request a copy of the Gibco™ Neurobiology Protocol Handbook at **thermofisher.com/neurohandbook**

Table 12. Media systems and reagents for differentiation.

	Ectoderm			Mesoderm	Endoderm
Application	NSC differentiation	Neuron differentiation	Dopaminergic neuron differentiation	Cardiomyocyte differentiation	Definitive endoderm differentiation
Media system	PSC Neural Induction Medium	CultureOne Supplement with B-27 Supplements	PSC Dopaminergic Neuron Differentiation Kit	PSC Cardiomyocyte Differentiation Kit	PSC Definitive Endoderm Induction Kit
Substrate	Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	Laminin Mouse Protein, Natural	Vitronectin (VTN-N) Recombinant Human Protein, Truncated Laminin Mouse Protein, Natural	Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	Vitronectin (VTN-N) Recombinant Human Protein, Truncated
Protocol duration	7 days	7–14+ days	35 days	14 days	2 days
Cell type generated	Neural stem cells	General or subtype neurons	Midbrain dopaminergic neurons	Cardiomyocytes	Definitive endoderm
Media format	50X supplement/500 mL basal, serum-free	Serum-free	Serum-free	Ready-to-use, xeno-free	Ready-to-use, xeno-free
Recommended characterization tool	Human Neural Stem Cell Immunocytochemistry Kit	HuC/HuD Monoclonal Antibodies for quantitative image analysis	Human Dopaminergic Neuron Immunocytochemistry Kit	Human Cardiomyocyte Immunocytochemistry Kit	NA

Need help differentiating your cells?

Thermo Fisher Scientific employs a dedicated team of stem cell scientists to help you achieve your project goals. See the section starting on **page 58** for all of our stem cell services.

PSC Neural Induction Medium

A streamlined path to neural differentiation

Gibco PSC Neural Induction Medium is a serum-free medium that provides high-efficiency neural induction of human PSCs (Figure 27) in only 7 days. Unlike existing methodologies, use of PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation, which adds time, labor, and variability (Figure 28). High-quality NSCs generated using PSC Neural Induction Medium have high expression of NSC markers and can be cryopreserved, expanded, and further differentiated into other neural cell types (Figure 29).

Find out more at **thermofisher.com/nscdiff**

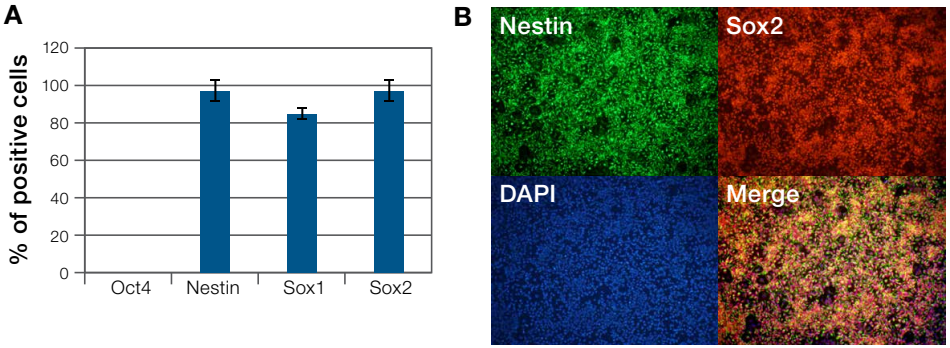


Figure 27. NSCs generated using PSC Neural Induction Medium express high levels of the NSC markers nestin, Sox1, and Sox2, and low levels of the residual pluripotent marker Oct4. (A) 80–90% neural induction efficiency. **(B)** Immunocytochemistry staining images of relevant NSC markers.

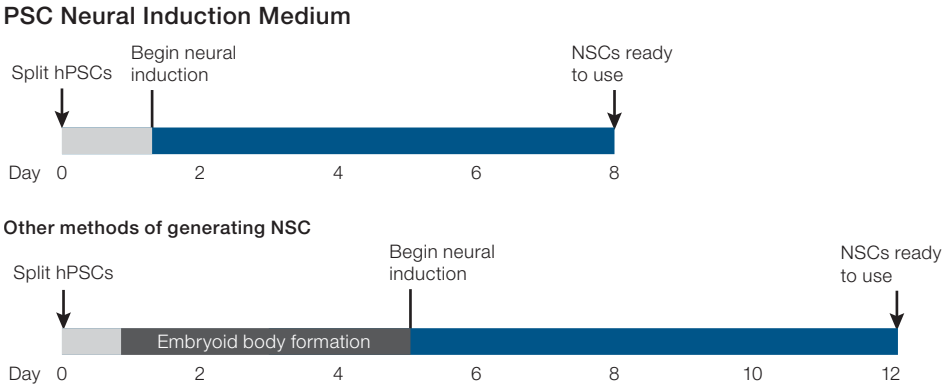


Figure 28. Unlike existing methodologies, PSC Neural Induction Medium does not require the intermediary step of EB formation, which adds time, labor, and variability.

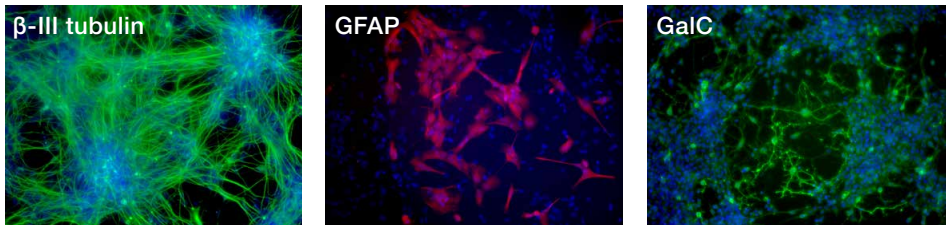


Figure 29. Neural stem cells generated using PSC Neural Induction Medium have high expression of NSC markers and can be further differentiated into other neural cell types.

CultureOne Supplement

Superior neuronal cell cultures

Gibco™ CultureOne™ Supplement significantly improves the differentiation of NSCs to neurons. As compared to conventional differentiation methods where NSCs can overgrow and become burdensome, use of CultureOne Supplement eliminates more than 75% of contaminating neural progenitor cells with minimal cell death and no effect on other kinase-mediated pathways. The resulting superior neuronal cell cultures of evenly distributed, differentiated neurons enable improved downstream assays, accelerated neuronal maturation, and seamless maintenance for 5 weeks or more (Figure 30).

Find out more at **thermofisher.com/cultureone**

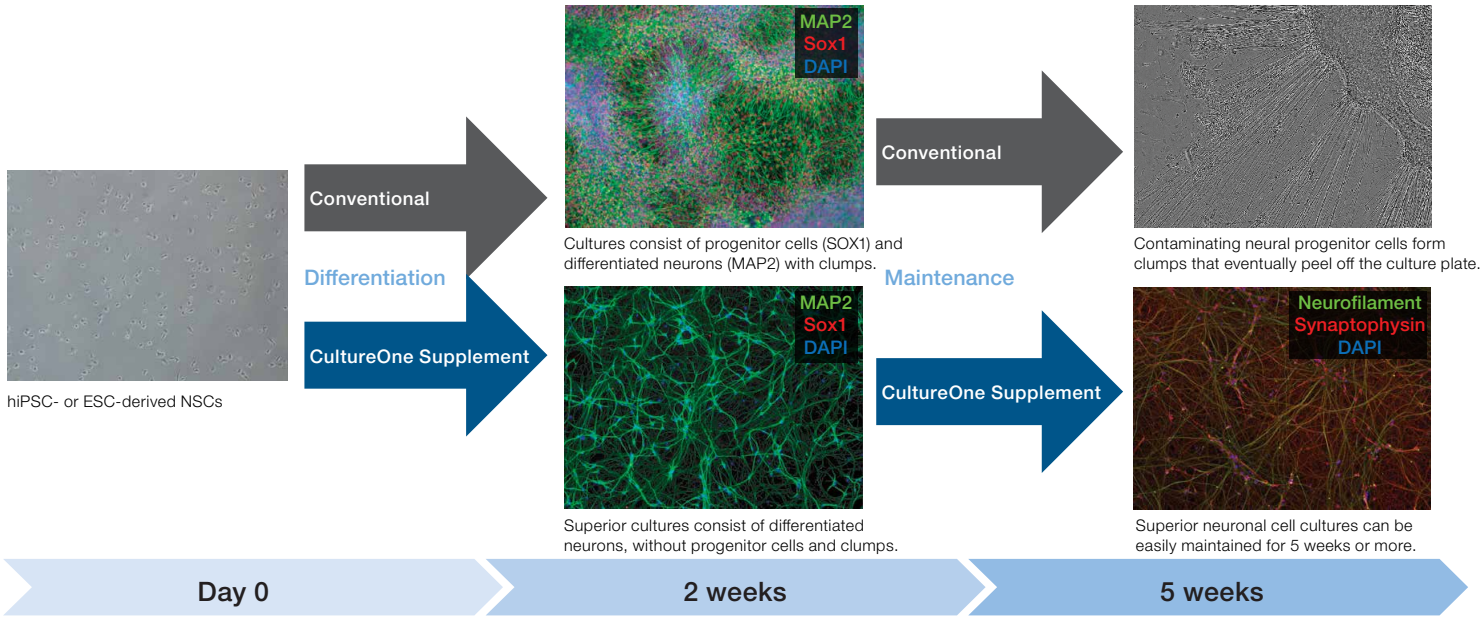


Figure 30. H9 ESC-derived NSCs were plated at a density of 5 x 10^4 cells/cm^2. Without CultureOne Supplement, cells at 2 weeks of differentiation were highly dense, formed cell clumps, and contained MAP2-positive neurons and a significant number of Sox1-positive NSCs. At 2 weeks of differentiation, cultures treated with CultureOne Supplement had an even distribution of MAP2-positive neurons with minimal Sox1-positive NSCs and no cell clumps. At 5 weeks of differentiation, differentiated cells treated with CultureOne Supplement expressed mature neuronal markers, neurofilament, and synaptophysin, and exhibited higher spike rates than conventional differentiation methods as measured by microelectrode array (MEA).

B-27 plus neuronal culture system

The most cited neural cell culture system consists of Gibco™ B-27™ Supplement and Gibco™ Neurobasal™ Medium. Originally optimized for long-term culture of rat hippocampal and cortical neurons, this combination has been shown, over two decades of research, to be suitable for a wide range of other neural applications including PSC-derived NSCs and neurons.

However, as the desire for more reliable and biologically relevant models has increased, so too has the necessity for a next-generation media system that can maintain and mature optimal densities of functional neurons over longer periods of time in vitro. The Gibco™ B-27 Plus Neuronal Culture System features an optimized formulation, upgraded manufacturing process, and more stringent quality control for raw materials and final product. These improvements enable increased neuronal survival by more than 50%, accelerated neurite outgrowth, improved electrophysiological activity, and maturation of neurons (Figure 31).

Learn more at **thermofisher.com/b27plus**

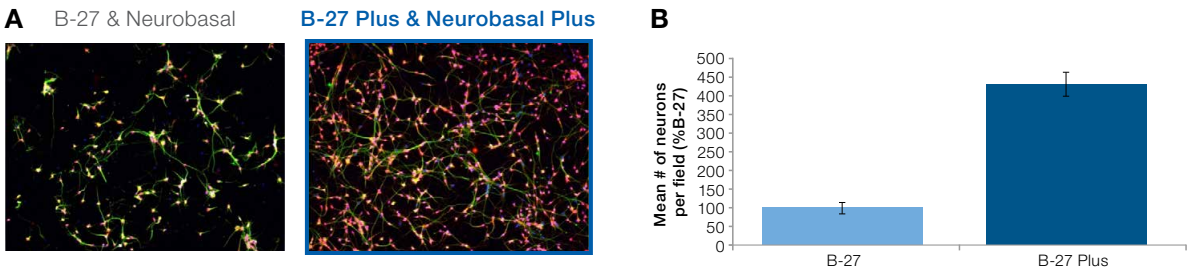


Figure 31. B-27 Plus Neuronal Culture System enables superior survival of human stem cell–derived neurons. Cryopreserved HIP Neurons (MTI-GlobalStem) were thawed in classic Neurobasal Medium with B-27 Supplement and plated onto polyethyleneimine-coated 96-well plates into two volumes of the listed media. Neurons were maintained for 4 weeks with half fluid changes two times per week. **(A)** Neurons were immunostained with neuronal dendritic marker, MAP2 (green), neuronal cell body marker, HuC/D (red), and nuclei were counterstained with DAPI (blue). **(B)** Comparability studies indicate that the B-27 Plus Neuronal Culture System is a significantly superior medium compared to the classic B-27–supplemented Neurobasal Medium, with improved neuronal survival and health in long-term cultures.

PSC Dopaminergic Neuron Differentiation Kit

Differentiate iPSCs to functional midbrain dopaminergic neurons

The Gibco™ PSC Dopaminergic Neuron Differentiation Kit enables the differentiation of PSCs to midbrain dopaminergic neurons. Unlike other protocols or commercially available solutions to differentiate PSCs to dopaminergic neurons, which can be biologically restrictive, lengthy, or ill-defined, the PSC Dopaminergic Neuron Differentiation Kit allows you to differentiate PSCs to dopaminergic neurons with increased flexibility, speed, and scalability, all while retaining proper biological relevance. The system uses a three-step approach to (1) specify hPSCs to midbrain floor plate cells, (2) expand and cryopreserve specified cells, and (3) revive and mature cells to midbrain dopaminergic neurons (Figures 32 and 33).

Find out more at **thermofisher.com/dopadiff**

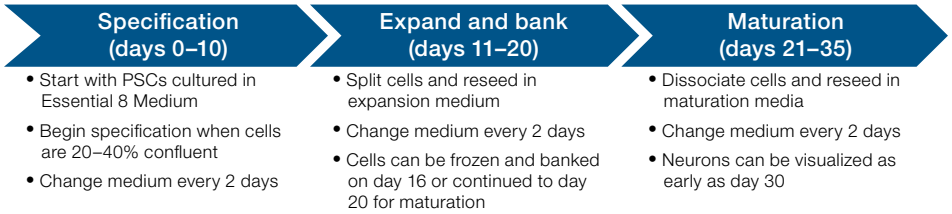


Figure 32. Pluripotent stem cells cultured in Essential 8 Medium. PSCs can be specified to the midbrain floor plate, expanded, and banked, then matured to midbrain dopaminergic neurons in 35 days. Floor plate–derived midbrain progenitors can be expanded up to 10 passages.

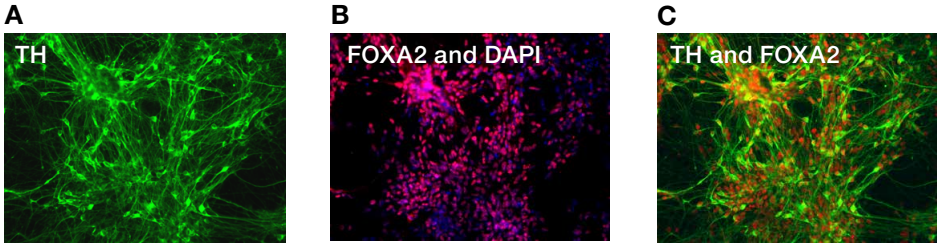


Figure 33. Representative images of mature dopaminergic neurons. The images were obtained from cells stained with reagents provided in the Invitrogen™ Human Dopaminergic Neuron Immunocytochemistry Kit after 14 days of maturation of floor plate progenitor cells in Dopaminergic Neuron Maturation Medium. The majority of the TH-expressing neurons also coexpressed FOXA2. **(A)** Anti-TH (green); **(B)** anti-FOXA2 (red) and Invitrogen™ NucBlue™ reagent (a DAPI nuclear DNA stain) (blue); and **(C)** merged image with anti-TH and anti-FOXA2 (green and red).

PSC Cardiomyocyte Differentiation Kit

Three simple steps. One simple kit.

The Gibco™ PSC Cardiomyocyte Differentiation Kit consists of a set of serum-free and xeno-free media that enable efficient differentiation of human PSCs to contracting cardiomyocytes in as few as 8 days. Unlike other methods that require multiple components and longer assay duration, the PSC Cardiomyocyte Differentiation Kit can be used to generate cardiomyocytes from PSCs in a ready-to-use media format and in less time (Figure 34).

Composed of three 1X media that require no thawing or mixing, each medium is used consecutively over a total of 14 days, resulting in functional cardiomyocytes that express relevant physiological markers (Figure 35), contract in culture, and can be subsequently maintained in culture for more than 15 days.

Find out more at **thermofisher.com/cardiacdiff**

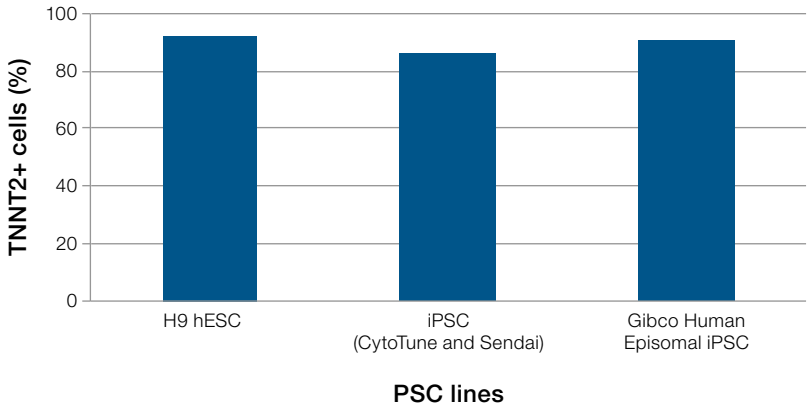


Figure 34. Efficiency across multiple PSC lines. PSC lines dissociated with Gibco™ TrypLE™ Express Enzyme were seeded at specific density onto a Geltrex-coated surface and cultured in Essential 8 Medium. After three days of expansion, PSC lines at optimal confluency were induced using the PSC Cardiomyocyte Differentiation Kit according to protocol and cultured for two weeks. Cells were harvested and analyzed for TNNT2 expression by flow cytometry. Results showed high cardiomyocyte differentiation efficiency among all lines when it reached optimal confluency at time of induction.

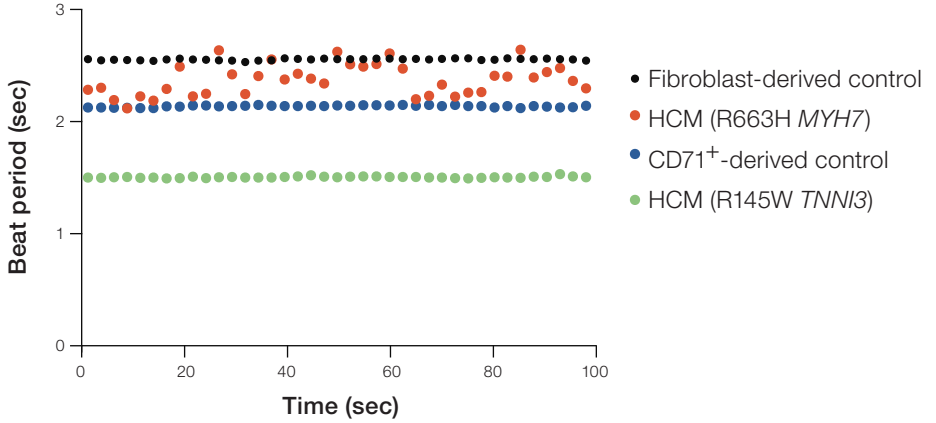


Figure 35. Electrophysiological assessment of hypertrophic cardiomyopathy patients' iPSC-derived cardiomyocytes generated using the PSC Cardiomyocyte Differentiation Kit on the Maestro™ Multielectrode Array (MEA) platform (Axion Biosystems). The arrhythmic beating of the cardiomyocytes with mutation is evident when comparing their beat period to those of cardiomyocytes derived from the other cell lines.

PSC Definitive Endoderm Induction Kit

Definitive endoderm cells in 48 hours

The Gibco™ PSC Definitive Endoderm Induction Kit consists of two xeno-free media that enable efficient induction of human PSCs to definitive endoderm. Unlike other methods that require multiple components and take 5 or more days, the PSC Definitive Endoderm Induction Kit enables you to generate ≥90% CXCR4⁺/PDGFRα⁻ definitive endoderm cells with only 2 components in just 2 days (Figures 36 and 37).

Each medium is supplied as a 1X complete medium, requiring no mixing of additional components, and the resulting definitive endoderm shows more than 90% high expression of the key markers Sox17 and FoxA2 across multiple PSC lines (Figure 38) and are capable of differentiating to downstream lineages.

See the complete set of data at **thermofisher.com/dendo**

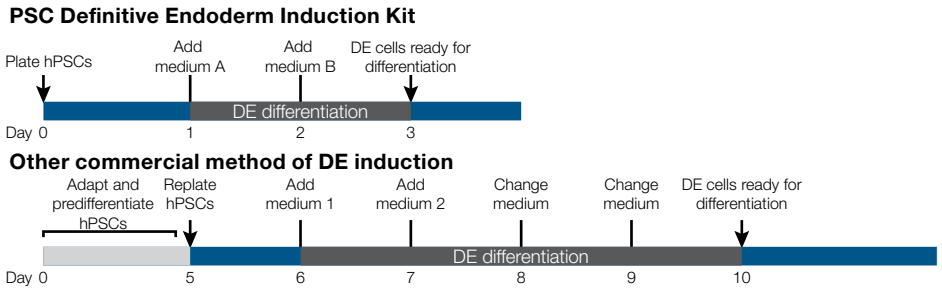


Figure 37. Compared to other differentiation protocols, the PSC Definitive Endoderm Induction Kit produces cells in up to 50% less time and requires no predifferentiation or mixing of media.

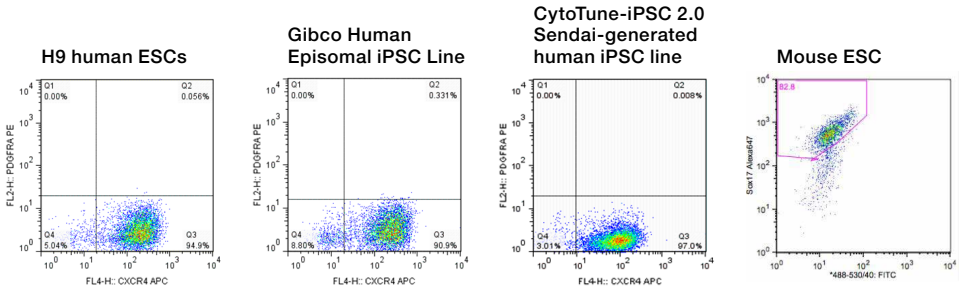


Figure 36. The PSC Definitive Endoderm Induction Kit produces definitive endoderm (DE) populations with high efficiency across hESC, hiPSC, and mESC lines. hiPSCs tested include cell lines reprogrammed using episomal vectors or the CytoTune kit. Representative dot plots for hESCs and hiPSCs show CXCR4⁺/PDGFRα⁻ cell populations derived from various cell lines. Representative dot plot for mESCs shows a SOX17⁺ cell population. For each experiment, unstained cells were used to set gates.

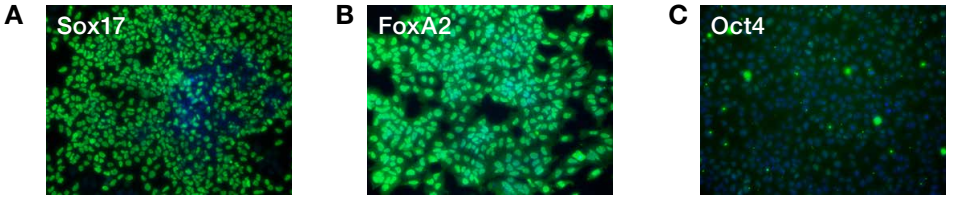
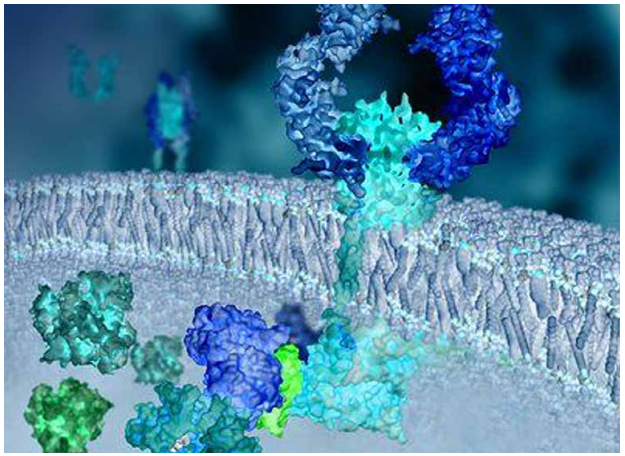


Figure 38. Immunocytochemistry of hESCs treated with the PSC Definitive Endoderm Induction Kit. At day 3, induced cells were immunostained for the endodermal transcription factors **(A)** Sox17 and **(B)** FoxA2, and the pluripotent marker **(C)** Oct4. Nuclei were counterstained with DAPI (blue) to assess total cell numbers.

Differentiation growth factors

Growth factors can stimulate stem cell differentiation and influence the stem cell developmental fate. High-quality Gibco™ growth factors are designed to give you high biological activity, high purity, and low endotoxin levels. Our growth factors are verified with Gibco™ media to have proven compatibility.



Fibroblast growth factor basic (bFGF, FGF-basic, FGF-2)
This large FGF protein family is involved in many aspects of development, including cell proliferation, growth, and differentiation. FGF-basic is a critical component for maintaining embryonic stem cells in culture in an undifferentiated state.

Epidermal growth factor (EGF)
EGF has a profound effect on the differentiation of specific cells *in vivo* and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin.

Granulocyte-macrophage colony-stimulating factor (GM-CSF)
GM-CSF is involved in many biological responses, including the growth and development of granulocyte and macrophage progenitor cells, stimulation and the initiation of differentiation of myeloblasts and monoblasts, and chemotaxis of eosinophils.

Activin A
Activin A is involved in multiple biological processes, including hematopoeisis, neural development, and inflammation.

Tumor necrosis factor (TNF)
TNF causes cytolysis and cytostasis of many tumor cell lines. TNF has a wide spectrum of activities, including chemotaxis of neutrophils, alteration of the endothelium, inhibition of anticoagulatory mechanisms, and promotion of angiogenesis.

Vascular endothelial cell growth factor (VEGF)
VEGF exerts angiogenic, mitogenic, and vascular permeability–enhancing activities specific for endothelial cells. VEGF has also been shown to be chemotactic for monocytes and osteoblasts.

Explore all Gibco growth factors at **thermofisher.com/growthfactors**

Characterization and analysis tools

Stem cell research requires cellular and molecular tools to confirm pluripotency or to help determine the utility of cells in downstream experiments. Whether analyzing proliferation, protein levels, gene expression, or epigenetic profiles, we have the right instruments, products, and services for your research.

Choose among the tools and services for stem cell analysis at **thermofisher.com/stemcellanalysis**

Types of characterization and analysis tools

Labeling and detection tools

Research products for studying stem cell structure, tracing and tracking stem cells, and analyzing proliferation, viability, and function

- Invitrogen™ Qdot™ nanocrystals
- Invitrogen™ Alexa Fluor™ dyes
- Invitrogen™ Alexa Fluor™ secondary antibodies and streptavidin
- Invitrogen™ primary antibodies
- Invitrogen™ Alkaline Phosphatase Live Stain
- Invitrogen™ cell health assays

Protein analysis

High-quality, easy-to-use reagents and kits for quantifying proteins, along with colorimetric and fluorimetric solution assays

- Applied Biosystems™ TaqMan® protein analysis assays
- Invitrogen™ multiplex assays
- Invitrogen™ antibodies for western detection
- Invitrogen™ ELISA kits
- Invitrogen™ western blotting kits

Sample preparation

Scalable, efficient nucleic acid and protein purification technologies, plus gene expression analysis tools

- Applied Biosystems™ protein expression sample preparation kits
- Invitrogen™ TaqMan® PreAmp Cells-to-C_T™ Kit
- Invitrogen™ RNA extraction and purification kits
- Invitrogen™ DNA purification kits

Genomic analysis

Trusted RT-qPCR, sequencing, and microarray platforms for a wide variety of genomic analyses

- Applied Biosystems™ TaqMan® hPSC Scorecard Panel
- Applied Biosystems™ AuthentiFiler™ PCR Amplification Kit
- PluriTest™-compatible Applied Biosystems™ PrimeView™ Global Gene Expression Profile Assays
- Applied Biosystems™ KaryoStat™ Assays
- Applied Biosystems™ TaqMan® Gene Expression Assays
- Applied Biosystems™ TaqMan® miRNA Assays
- Applied Biosystems™ TaqMan® SNP assays
- Applied Biosystems™ TaqMan® Copy Number Assays
- Ion AmpliSeq™ panels
- Ion Torrent™ Oncomine™ assays

Characterization tools for pluripotent stem cells

Verifying the quality of your PSCs is critical to moving your research goals forward. We have a variety of cellular and molecular methods to help you completely and cost-effectively characterize your PSCs. Whether you're looking to identify PSCs, confirm pluripotency, evaluate trilineage differentiation potential, or verify genomic stability, we have the tools you need to characterize your lines with confidence.

Find the right assay for your research at **thermofisher.com/characterization**

Table 13. Characterization products overview.

	Easy identification of pluripotency without compromising cell integrity	Specific and flexible identification of PSCs	Cost-effective global confirmation of pluripotency marker expression	Pluripotency evaluation and trilineage differentiation potential confirmation	Array-based alternative to G-banding karyotyping
Product name	**Alkaline Phosphatase Live Stain**	**PSC immunocytochemistry kits**	**PluriTest-compatible PrimeView Global Gene Expression Profile Assays**	**TaqMan hPSC Scorecard Panel**	**KaryoStat and KaryoStat HD Assays**
How specific are the results?	Low (stains mouse and human stem and progenitor cells)	Medium (stains human ESCs and iPSCs)	High (whole-transcriptome gene expression profile)	High (profiles expression of human PSCs and early germ layer markers)	High
Will the cells remain viable?	Yes	No	No	No	No
How long before I see results?	20 minutes or less	90–120 minutes	2 days	6–8 hours	3–4 days
Are data analysis tools included?	No	No	Yes, free online PluriTest analysis tool	Yes, free cloud-based software	Yes, free downloadable Chromosome Analysis Suite (ChAS) software
Is a reference standard included?	No	No	Yes	Yes	No
Are EVOS cell imager protocols available?	Yes	Yes	No	No	No
Training and expertise required	Minimal	Minimal	Moderate	Moderate	Moderate to high
Unit size	500 µL vial sufficient for staining twelve 6 cm dishes	100 µg	30 arrays (one sample/array) or one 16-sample array plate	One 384-well plate kit (4 samples/plate) or two 96-well plates (1 sample/plate)	24 arrays (one sample/array)

Need help characterizing your cells?

Thermo Fisher Scientific employs a dedicated team of stem cell scientists to help you achieve your project goals. See the section starting on **page 58** for all of our stem cell services.

TaqMan hPSC Scorecard Panel

Quantitative analysis of trilineage differentiation potential

The TaqMan hPSC Scorecard Panel assesses trilineage differentiation potential using real-time PCR assays and intuitive data analysis software. The hPSC Scorecard assay was developed in collaboration with Alexander Meissner and follows his landmark publication [2].

The assay offers:

- A quantitative and time-saving alternative to teratoma formation [3]
- Comparison of expression profiles to a reference standard
- An easy-to-use platform with pre-plated assays and dedicated, intuitive analysis software

Find out more about this innovative technology at **thermofisher.com/scorecard**

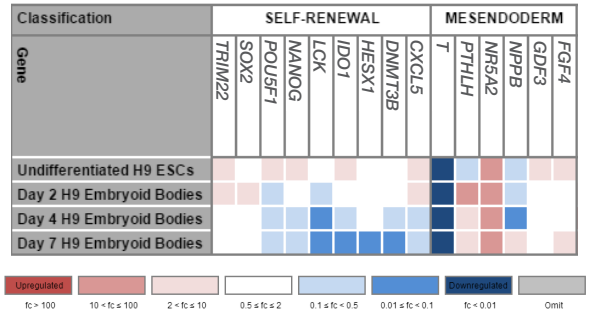


Figure 39. Gene expression results for self-renewal and germ layer markers are summarized in an easy-to-read format.

PrimeView Global Gene Expression Profile Assays

Confirm pluripotency

PluriTest-compatible PrimeView Global Gene Expression Profile Assays enable quick and cost-effective verification of pluripotency profiling.

The assays offer:

- Compatibility with the PluriTest Online Analysis Tool, a published and established method with over 16,000 samples analyzed
- More than 36,000 transcripts and variants are compared against an extensive reference set of more than 450 samples
- A free and simple-to-use cloud-based analysis tool

Find out more about pluripotency verification at **thermofisher.com/primeview**

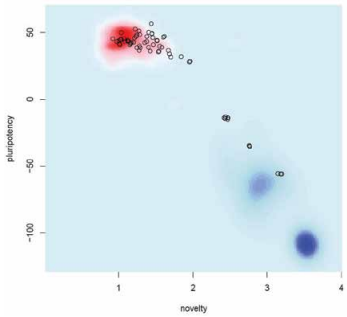


Figure 40. The pluripotency plot is an output of the PluriTest Online Analysis Tool and is a visual representation of the pluripotent and nonpluripotent samples in the analysis. The red and blue background hint at the empirical distribution of the pluripotent (red) and nonpluripotent samples (blue) in the reference data set.

KaryoStat Assays

Verify genomic stability

The KaryoStat Assay and the Applied Biosystems™ KaryoStat™ HD Assay provide a cost-effective alternative to G-banding karyotyping, offering accurate genotyping (sample ID) and whole-genome coverage for accurate detection of stem cell lines with chromosomal abnormalities.

The assays offer:

- Accurate detection of chromosomal abnormalities
- Karyotyping and genotyping (sample ID) with a single assay
- Simple analysis tool that does not require cytogenetic expertise
- Results in 3–4 days

Find out more about our G-banding karyotyping alternative at **thermofisher.com/karyostat**

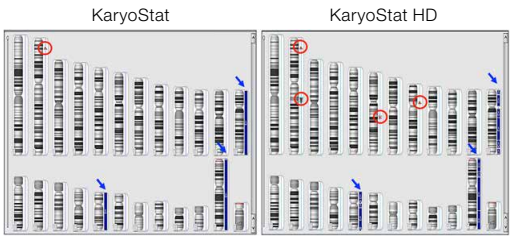


Figure 41. The KaryoStat Assay (left) and KaryoStat HD Assay (right) detect trisomy for chromosomes 12, 17, and X in BG01V, a human embryonic stem cell line with abnormal karyotype. In addition, both assays detect a loss on chromosome 2 that was not detected by G-banding karyotyping.

The use of antibodies to characterize pluripotent stem cells

The Thermo Fisher Scientific portfolio includes hundreds of thousands of antibodies that are extensively verified and specific, including many that can be used for characterizing stem cells. For example, c-Myc and Oct4 are transcription factors expressed in iPSCs, and are also abundantly expressed across a wide variety of cell lines. Consistent with this biology, Invitrogen™ c-Myc antibody detects a single band with the expected molecular size in western blot (WB) analysis across different cell lines tested (Figure 39A). Immunocytochemical (ICC) analysis using this antibody shows the expected nuclear localization (Figure 39B). The specificity of Invitrogen™ Oct4 antibody is demonstrated by siRNA-mediated knockdown of Oct4 in a WB (Figure 39C). This antibody also performs well in chromatin immunoprecipitation (ChIP), which is a relevant application (Figure 39D).

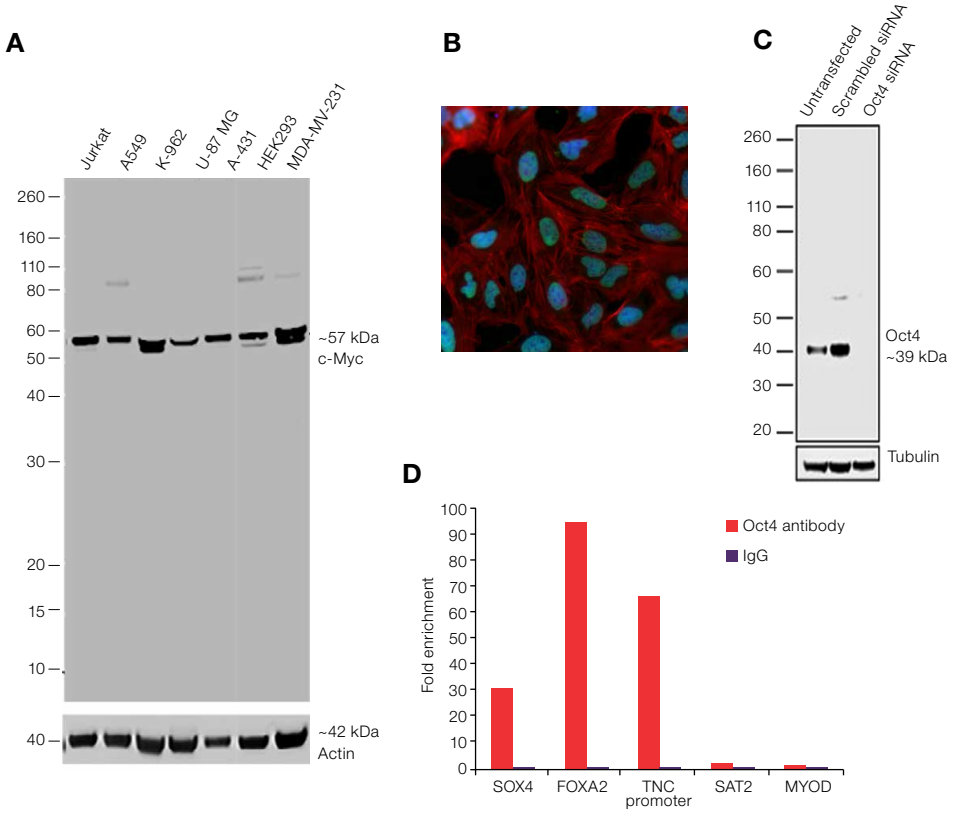


Figure 42. Antibodies against pluripotency markers. Functional validation of c-Myc antibody in **(A)** a WB and **(B)** ICC (antigen labeled in green and phalloidin in red). **(C)** Specificity of Oct4 antibody in a WB demonstrated by siRNA-mediated knockdown. **(D)** ChIP using Oct4 antibody.

Selected instruments for stem cell characterization and analysis

Invitrogen™ EVOS™ M7000 Imaging System

Perfect for long-term live-cell imaging, image tiling and stitching, and a broad range of automated imaging applications. With our wide selection of reagents and kits for labeling and detection, including our flagship Invitrogen™ Alexa Fluor™ dyes, you will obtain truly stunning images.

Determine which cell imaging system is right for you at **thermofisher.com/evos**

Attune NxT Flow Cytometer and Autosampler

The Invitrogen™ Attune™ NxT Flow Cytometer with superior speed and clog-resistant engineering is configurable with up to 4 lasers and 6–16 parameters. Convert between tubes and plates in seconds, and leverage complete walk-away automation of your 96- or 384-well plates with the robotic automation–capable Invitrogen™ Attune™ NxT Autosampler.

See more at **thermofisher.com/attune**

Countess II FL Automated Cell Counter

With options for a reusable slide and fluorescence capabilities—brightfield and two user-changeable fluorescence channels—the Invitrogen™ Countess™ II FL Automated Cell Counter can count cells and measure cell viability in as little as 10 seconds.

Find out more at **thermofisher.com/countess**

QuantStudio real-time PCR (qPCR) family

Flexibility. Versatility. Connectivity. Speed. Precision. Everyone's needs are unique, and that's why we have expanded the Applied Biosystems™ QuantStudio™ family of real-time PCR and digital PCR systems.

Now you can pick the qPCR platform that best fits your research requirements—find your fit today at **thermofisher.com/quantstudio**

Ion Personal Genome Machine (PGM) System

Powered by Ion Torrent™ semiconductor chip technology, the Ion Personal Genome Machine™ (PGM™) sequencer delivers the fastest sequencing run times, at the most affordable price, of any benchtop sequencer.

See more at **thermofisher.com/pgm**

GeneChip Scanner 3000 7G System

The Applied Biosystems™ GeneChip™ instrument system is a fully integrated platform for conducting your research using GeneChip-brand probe arrays. The Applied Biosystems™ GeneChip™ Scanner 3000 7G System allows you to scan next-generation higher-density sequences for SNP, copy number, and expression arrays that can interrogate over 6 million unique sequences.

Find out how at **thermofisher.com/genechipscanner**

Ion GeneStudio S5 systems

The Ion GeneStudio™ S5 series of instruments is flexibly designed to enable a broad range of targeted NGS applications with industry-leading speed and scalability. Select from five different sequencing chips to sequence a throughput range from 2 million to 130 million reads per run. Simply choose the chip size and the instrument that matches your throughput and application needs.

See how at **thermofisher.com/genestudio**

GeneTitan MC Instrument

Transform your lab with the superior power of streamlined array processing for discovery, exploration, and screening.

The Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument for expression and genotyping seamlessly integrates hybridization, washing, and imaging to provide automated array processing—whether you are performing basic or applied research.

See more at **thermofisher.com/genetitan**

Cell therapy systems

Regardless of where you are in your cell therapy development, Thermo Fisher Scientific has solutions to help you achieve your cell therapy goals—all the way through to commercialization. Our extensive portfolio of xeno-free and animal origin–free media support cost-effective basic research; and when you're ready to transition your cell therapy to the clinic, our complementary Gibco™ Cell Therapy Systems (CTS™) formulations are designed to enable clinical and commercial cell therapy manufacturing according to Good Manufacturing Practice (GMP). CTS media and reagents undergo extensive quality and safety testing and have a high degree of regulatory documentation and support, including Certificates of Analysis, Certificates of Origin, and Drug Master Files or regulatory support files. Our goal is to ease the burden on your quality systems by helping to support your regulatory submission and minimize risk throughout.

Find the best solutions and support for your PSC therapy needs at **thermofisher.com/ctsstemcells**

Support resources

- Download the cell therapy solutions brochure at **thermofisher.com/celltherapysolutions**
- See the cell therapy product selection guide at **thermofisher.com/cell-gene-therapy-tool**
- Access the CTS mini-documentary series videos at **thermofisher.com/cts-videoseries**

cGMP-compliant manufacturing

- Manufactured in conformity with GMP and follow USP <1043>* and Ph Eur 5.2.12 regulations
- Internal manufacturing sites are US Food and Drug Administration (FDA)–registered, ISO 13485–certified, and regularly audited

Testing and documentation

- Traceability documentation, including Drug Master Files and regulatory support files, and certificates of origin
- Product safety testing—including sterility, endotoxin levels, and the presence or absence of mycoplasmas on applicable products

Proven use

- Used in FDA-approved and EMA-approved CAR T therapies [4,5] and the first FDA-approved therapeutic cancer vaccine [6]
- Used in over 100 clinical trials

* CTS products are manufactured to meet the ancillary material responsibilities for cell-, gene-, and tissue-engineered products. Other aspects of USP <1043> are the responsibility of the end user to assess. Thermo Fisher Scientific cannot fulfill USP <1043> in regard to application and therapy-specific aspects (e.g., use in a finished therapeutic, assessment of removal from a finished therapeutic, and possibly biocompatibility, cytotoxicity, or adventitious agent testing).

Pluripotent stem cell therapy workflow solutions

Isolation

Isolation

(T cells) CTS Dynabeads CD3/CD28

$CD34^+$ isolation beads

Medium

(T cells) CTS OpTmizer medium

Dissociation and passaging reagent

CTS TrypLE Select Enzyme

Reprogramming

Reprogramming kit

CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit

Transfection reagents

(Fibroblasts) Lipofectamine Stem Transfection Reagent

Lipofectamine 3000 Transfection Reagent

Electroporation

(T cells) Neon Transfection System

Banking and recovery

Cryopreservation

CTS Synth-a-Freeze Medium

CTS PSC Cryopreservation Kit

CTS PSC Cryomedium

Recovery

CTS RevitaCell Supplement

CTS Essential 8 Medium and CTS Vitronectin

rhLaminin-521

Expansion and gene editing

Expansion media systems

CTS KnockOut SR XenoFree Medium

CTS KnockOut DMEM/F-12

CTS KnockOut DMEM

CTS Essential 8 Medium and CTS Vitronectin

CTS Versene Solution

Custom media and services

Genome editing

TrueCut Cas9 Protein v2

TrueGuide Synthetic gRNAs

TrueDesign Genome Editor

TrueTag Donor DNA Kits

Single-use technologies

BioProcess Containers (BPCs)

Transfer assemblies

Static bags

Rocker bags

Differentiation

Differentiation reagents

PSC Cardiomyocyte Differentiation Kit

PSC Dopaminergic Neuron Differentiation Kit

PSC Definitive Endoderm Induction Kit

CTS N-2 Supplement

CTS KnockOut DMEM/F-12

CTS Neurobasal Medium

CTS Neurobasal-A Medium

CultureOne Supplement

CTS Essential 6 Medium

Wash, fill, finish, and cryo

Wash

CTS Dulbecco's Phosphate Buffered Saline

Cryopreservation medium

CTS Synth-a-Freeze Medium

Cryogenic storage and logistics

Banking services

Cold-chain logistics solutions

Find the best solutions and support for your pluripotent stem cell therapy needs at **thermofisher.com/ctsstemcells**

CTS Essential 8 Medium

Fully defined human PSC culture medium for clinical research applications

Based on the widely published Essential 8 Medium, Thermo Fisher Scientific has developed a Gibco™ Cell Therapy Systems (CTS™)-grade, fully defined human PSC culture medium. Gibco™ CTS™ Essential 8™ Medium offers all of the same benefits and performance of the research-use product (Figure 43), with components not directly derived from animals to support clinical research applications.

Why CTS Essential 8 Medium?

- **Helps minimize risks**—fully defined, with components not directly derived from animals
- **Facilitates regulatory filings**—cGMP-manufactured and regulatory documentation available, including FDA Drug Master Files
- **Provides seamless transition**—same 8-component formulation as research-use Essential 8 Medium, but with components not directly derived from animals

Find out more about CTS Essential 8 Medium at **thermofisher.com/ctsessential8**

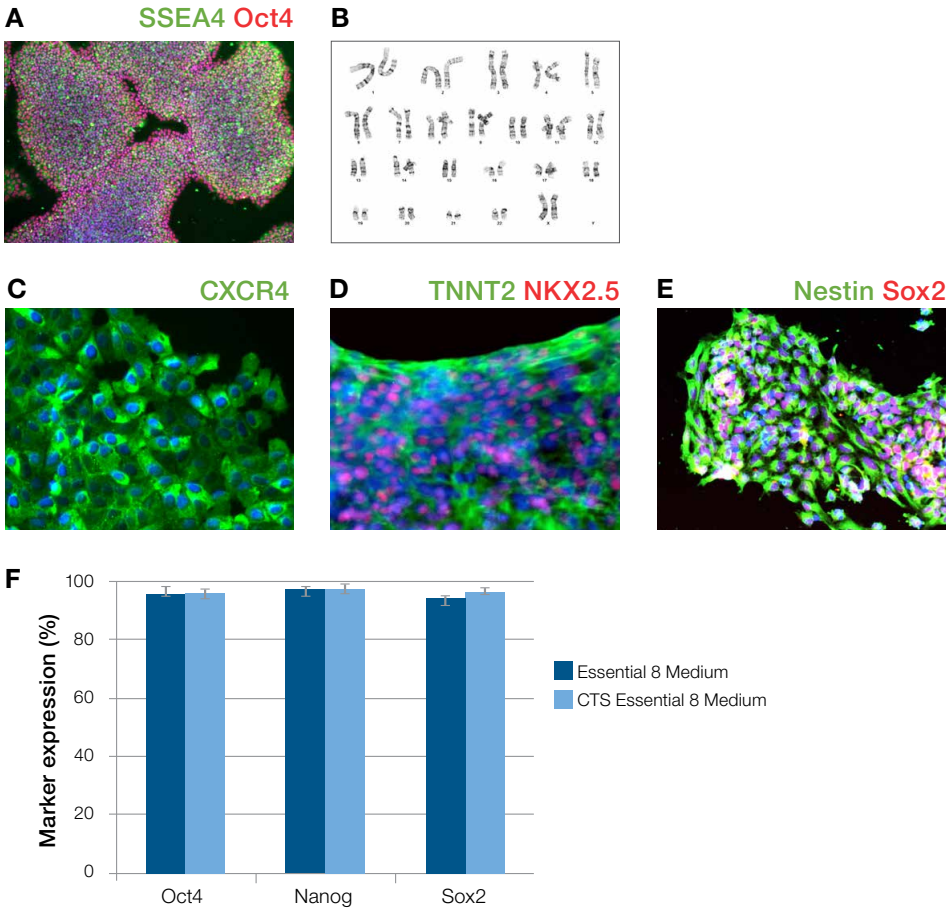


Figure 43. CTS Essential 8 Medium enables long-term PSC culture, trilineage differentiation, and a seamless transition from research-use Essential 8 Medium. PSCs cultured in CTS Essential 8 Medium for >30 passages **(A)** express PSC markers Oct4 and SSEA4 and **(B)** maintain normal 46, XX karyotype. PSCs cultured in CTS Essential 8 Medium are able to differentiate into the three germ layers, as exemplified by differentiation into **(C)** definitive endoderm, **(D)** cardiomyocytes, and **(E)** neural stem cells using the respective Gibco differentiation kits or induction media. **(F)** PSCs cultured in CTS Essential 8 Medium show PSC marker expression similar to that observed in research-use Essential 8 Medium, as measured by quantitative immunocytochemistry.

CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit

The first off-the-shelf reprogramming system for clinical research

The Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit is the first off-the-shelf reprogramming system designed for clinical and translation research and manufactured in accordance with GMP requirements. Like the CytoTune-iPS 2.0 Sendai Reprogramming Kit, this kit uses Sendai particles to deliver Yamanaka factors that are critical for efficient generation of induced pluripotent stem cells (iPSCs).

Why CTS CytoTune-iPS 2.1?

- Xeno-free workflow for generation of iPSC lines from both fibroblasts and blood for clinical research
- High-efficiency Sendai delivery of reprogramming factors
- Extensive testing and documentation to support your regulatory submission

Find out more at **thermofisher.com/cytotune**

CTS PSC Cryopreservation Kit

Cryopreservation and recovery of PSCs for clinical research

The Gibco™ CTS™ PSC Cryopreservation Kit consists of the Gibco™ CTS™ PSC Cryomedium and Gibco™ CTS™ RevitaCell™ Supplement. When used in combination, these reagents minimize the loss of cell viability and maximize post-thaw recovery.

Why the CTS PSC Cryopreservation Kit?

- **Provides efficient recovery**—routinely achieves higher viability than traditional methods of cryopreservation
- **Offers consistency**—media maintains normal morphology, pluripotency, and karyotype of PSCs
- **Reduces variability**—optimized reagents minimize the causes of genetic instability of PSC

Other recommended CTS products

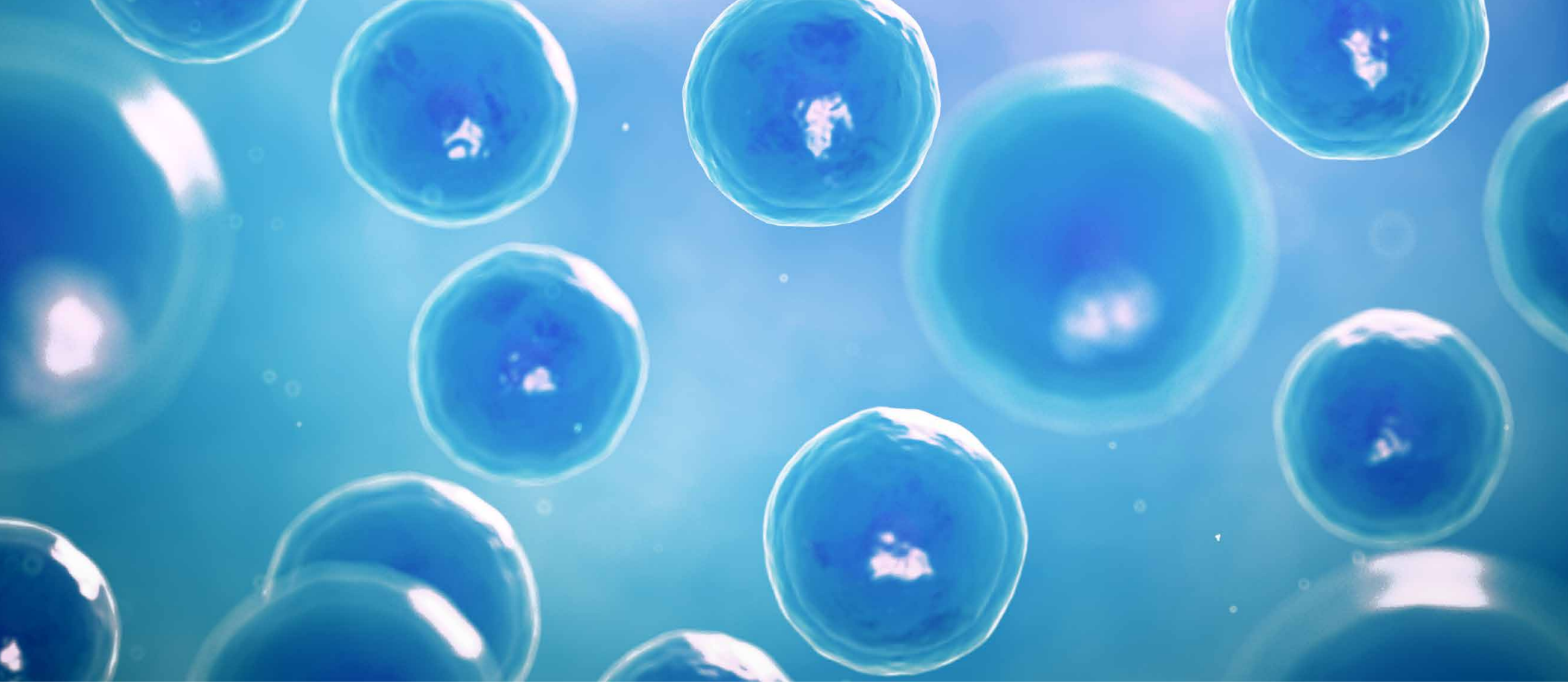
Gibco™ CTS™ Versene™ Solution

A gentle non-enzymatic cell dissociation reagent for use in routine clump passaging of PSCs while maintaining viability over multiple passages

Gibco™ CTS™ Essential 6 Medium

A xeno-free medium that provides a flexible format to suit different applications, including reprogramming and differentiation

Learn more about these and other solutions at **thermofisher.com/ipsctherapy**



Services and support

Built on the stem cell innovations that we have introduced throughout the past decade, the CellModel™ Services offered by Thermo Fisher Scientific enable stem cell scientists to reach their desired outcomes faster. We offer stem cell researchers choices at every stage of their research, including innovative tools that make it easier for you to do it yourself as well as a custom services offering that utilizes our experienced team of stem cell professionals to deliver your desired results.

CellModel Services workflow

We offer fully customizable services at every stage of the stem cell workflow as an extension of your lab. Choose the services that best fit your research needs.

Inquire about other services or instrumentation at **thermofisher.com/askdiscovery**

Sample

Reprogramming

Reprogram your human fibroblasts or blood cells to PSCs

Reprogram human fibroblasts or blood cells and provide PSCs to you in 4–6 months.

Choose from two non-integrating reprogramming technologies:

- CytoTune-iPS 2.0 Sendai Reprogramming Kit
- Invitrogen™ Epi5™ Episomal iPSC Reprogramming Kit

Engineering

Introduce disease-relevant mutations or indels into your PSCs

Custom-design stable stem cell lines using the most robust and reliable editing technologies that are commercially available.

Award-winning editing tools:

- Invitrogen™ GeneArt™ CRISPR-Cas9 products
- Invitrogen™ GeneArt™ Precision TALs

Modification options:

- Knockouts, SNPs
- Large knock-ins

Differentiation

Differentiate your PSCs to your desired terminal cell type

Stem cell differentiation projects are typically completed within 4–8 weeks from project initiation.

Available cell types:

- Dopaminergic neurons and progenitors
- Central nervous system (CNS) neurons and progenitors
- Cardiomyocytes
- Custom

Assay development

Develop and optimize custom assays to support drug discovery and development

A broad range of assay tools and instrumentation can be implemented with PSCs or their differentiated progeny to interrogate disease-relevant biology.

- Cell viability
- Cell proliferation
- Apoptosis
- Pathway signaling
- Gene and protein expression
- Posttranslational modifications
- Custom

Screening

Screen compounds using our high-throughput screening facility

We also offer stem cell screening services to enable rapid profiling of your compounds against many assay technology platforms through our SelectScreen™ team and services.

Characterization

Robust tools to characterize your PSCs, or differentiated cell types, anywhere throughout the process

Genomic analysis
- Applied Biosystems™ KaryoStat+ Karyotyping Service
- Ion Torrent™ Oncomine™ service
- KaryoStat and cell identification service

Pluripotency
- PluriTest™ Service
- Applied Biosystems™ TaqMan® hPSC Scorecard Service
- Tri-lineage differentiation service

Sterility
- Mycoplasma detection service

Note: We are continually expanding our service capabilities and offerings. Please reach out to your local sales specialist to see how we can help achieve your project goals.

CellModel Services—how can we help?

Why outsource?

There are many good reasons to outsource your stem cell projects. Outsourcing gives you:

- Access to new technology and specialized skill sets you might not have in-house
- The ability to free up your R&D resources to focus on other strategically important initiatives
- Focused resources to help accelerate your development timelines

How can we help you accomplish your stem cell goals?
Find out more at **thermofisher.com/cellmodels**

Advantages of working with our team for stem cell services include:

- **Expertise**—dedicated team of stem cell scientists to deliver results on your project
- **Full transparency**—detailed protocols provided to you after project completion to demonstrate how we reached each milestone and to document the tools we utilized
- **High-quality products**—all of the reagents and media used by our stem cell service can be purchased and used in your own lab to facilitate your post-service projects
- **Exceptional support**—experienced custom service project manager providing exceptional support and frequent communication

David Piper
Sr. Director, R&D

"Our customers really are the experts in the biology that they are studying, but as a tool provider, we have an intimate familiarity with the technology that can help our customers solve a biology problem."

"We can take cell-based or stem cell–based assays and configure not just large-scale provisioning of these cells, but we can transfer them directly into screening operations and seamlessly move our customers from an assay development paradigm into more of an operational screening exercise."

What our customers have to say:

"The services staff had a high level of expertise and a genuine interest in making sure that the project was successful. All personnel were highly knowledgeable and professional. My initial meetings and discussions set a very positive tone for the services and professionalism of Thermo Fisher Scientific."

"Our request was well organized, price points well explained, and [it was] shipped to us at a convenient time, avoiding the holiday period. The team was accommodating when we were unsure of our own MTA arrangements."

"Good/fast responsiveness. High-quality work of a competent team."

CellModel Services—case study

Andrew, a senior scientist, had some PSCs and wanted to create disease-relevant neuronal models to support his drug discovery research. Our team of dedicated stem cell scientists used Andrew's three PSC lines and stably integrated a Cas9 nuclease into the cells using lentivirus to easily edit the cell lines. Below is the research plan we created for Andrew.

PSC

Genome editing

Introduce CRISPR gRNAs and donor DNA into the Cas9 PSCs to drive homology-directed recombination and revert disease-relevant mutants to a "wild-type" genotype.

Stable Cas9 PSC lines

Culturing

After the "correction" of the disease-relevant mutations, the team expanded and cryopreserved the edited PSCs to generate a suitably sized bank of cells to support further R&D activities.

Cryopreservation of PSCs

Differentiation

The native and edited PSCs were differentiated and specified to midbrain floor plate progenitor cells using the PSC Dopaminergic Neuron Differentiation Kit. Progenitor cells were expanded and banked to support further differentiation to regionally specified dopaminergic neurons.

Specification to midbrain floor plate progenitors

Our CellModel Services were recently used to build disease models including a Parkinson's disease model and a cardiac disease model. Five-step, easy-to-follow workflows outlining the development of these models are available.

Learn more at **thermofisher.com/diseasemodels**

Pluripotent Stem Cell Education

Your journey to stem cell excellence begins with Gibco™ PSC Education. From textbook concepts to successful practices, this top-tier educational program offers a wide variety of tools and resources to enable your PSC research success.

Explore the modules below to access free-to-use handbooks, virtual trainings, webinars, and the expertise you need to gain confidence in the lab.

Culture your career at **thermofisher.com/pseducation**

Expand your knowledge with:

- PSC Resource Handbook
- PSC Protocol Handbook
- Neurobiology Protocol Handbook
- Gibco™ 5 Days of Stem Cells virtual event

Test your knowledge with:

- Gibco™ PSC digital training courses

Apply your knowledge with:

- How-to videos
- Protocols
- Application notes
- FAQs
- Technical support

Gibco training courses

Pluripotent stem cell digital training

Whether you are new to PSC research or need a refresher course, our digital training courses provide detailed, step-by-step stem cell training so you feel confident using stem cells in your research.

Developed by our stem cell experts, the self-guided digital courses are structured to virtually guide you through a variety of stem cell techniques. Each course includes written instruction, how-to videos, and interactive quizzes for you to test your knowledge.

Featured courses include:

- Generation of human induced pluripotent stem cells
- Thawing pluripotent stem cells
- Innovation in stem cell suspension culture
- Gene editing in pluripotent stem cells
- Cardiomyocyte differentiation
- Getting started in 3D cell culture
- Neural organoid generation from pluripotent stem cells
- 3D cell model characterization and analysis

All courses can be accessed for free at **thermofisher.com/pseducation**

Ordering information*

Product	Cat. No.
Somatic and progenitor cells	
CTS Immune Cell Serum Replacement**	A25961-01
Human Dermal Fibroblasts, Adult	C0135C
Human Dermal Fibroblasts, Neonatal	C0045C
StemPro-34 SFM	10639-011
StemPro BM Mesenchymal Stem Cells	A15652
StemPro Human Adipose-Derived Stem Cell Kit	R7788110
StemPro Human Adipose-Derived Stem Cells	R7788115
StemPro MSC SFM XenoFree	A10675-01
StemPro NSC SFM	A1050901
Reprogramming	
CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit (1 pack)**	A34546
CytoTune-iPS 2.0 Sendai Reprogramming Kit (1 pack)	A16517
CytoTune-iPS 2.0 Sendai Reprogramming Kit (3 pack)	A16518
Epi5 Episomal iPSC Reprogramming Kit	A15960
Episomal iPSC Reprogramming Vectors	A14703
Culture	
Collagenase IV	17104-019
CTS Essential 8 Medium**	A2656101
CTS KnockOut SR XenoFree Medium**	12618013
CTS RevitaCell Supplement**	A4238401
CTS TrypLE Select Enzyme**	A12859-01
Essential 8 Adaptation Kit	A25935
Essential 8 Flex Medium Kit	A2858501
Essential 8 Medium	A1517001
KnockOut DMEM	10829018
KnockOut Serum Replacement	10828-028
RevitaCell Supplement	A26445-01
StemFlex Medium	A3349401
StemPro Accutase Cell Dissociation Reagent	A1110501
StemPro EZPassage Disposable Stem Cell Passaging Tool	23181-010
StemPro hESC SFM	A10007-01
StemScale PSC Suspension Medium	A4965001
CO_2 Resistant Shaker	88881101
TrypLE Express Enzyme (1X), no phenol red	12604013
TrypLE Select Enzyme (1X), no phenol red	12563011

Product	Cat. No.
Matrices and feeder cells	
CTS Vitronectin (VTN-N) Recombinant Human Protein, Truncated**	A27940
CTS Versene Solution**	A4239101
Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	A1569601
Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A1413301
B6-Puro Mouse Embryonic Fibroblasts, Irradiated	A34965
CF1 Mouse Embryonic Fibroblasts, Irradiated	A34181
CF1 Mouse Embryonic Fibroblasts, MitC-Treated	A34959
C57BL/6 Mouse Embryonic Fibroblasts, MitC-Treated	A34962
CF6-Neo Mouse Embryonic Fibroblasts, Irradiated	A34963
CF6-Neo Mouse Embryonic Fibroblasts, MitC-Treated	A34964
DR4 Mouse Embryonic Fibroblasts, Irradiated	A34966
Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903
rhLaminin-521	A29248
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Cryopreservation	
CTS PSC Cryomedium**	A4238801
CTS PSC Cryopreservation Kit**	A4239301
CTS Synth-a-Freeze Cryopreservation Medium**	A13713-01
PSC Cryopreservation Kit	A2644601
Synth-a-Freeze Cryopreservation Medium	A12542-01
Cell culture plastics	
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom (125 mL)	4115-0125
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom (250 mL)	4115-0250
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom (500 mL)	4115-0500
Nunclon Sphera 96 U-Well Plate	174925
Nunclon Sphera 6-Well Plate, 24-Well Plate	174932, 174930
Nunc Lab-Tek II CC^2 Chamber Slide System, 1-, 2-, 4-, and 8-well	154739, 154852, 154917, 154941
Nunc Non-Treated Multidishes	150239, 144530
Nunc Poly-D-Lysine Collagen I Coated Multidishes	152035, 152025
Nunc Collagen I Coated Multidishes	152034
Nunc Multidishes with UpCell Surface, 6-, 12-, 24-, and 48-well	174901, 174900, 174899, 174898
Transfection	
Lipofectamine 3000 Transfection Reagent	L3000-008
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	CMAX00015
Lipofectamine MessengerMAX Transfection Reagent	LMRNA015
Lipofectamine Stem Transfection Reagent	STEM00008
Neon Transfection System	MPK5000S

Product	Cat. No.
Genome editing	
Cas9 iPSC	Contact GEMServices@thermofisher.com
Cas9 stable cell line	
GeneArt CRISPR Nuclease mRNA	A29378
GeneArt Genomic Cleavage Detection Kit	A24372
GeneArt Precision gRNA Synthesis Kit	A29377
Introduction to CRISPR-Cas9 Genome Editing Hands-On Workshop	A33133
LentiArray Cas9 Lentivirus, 100 µL	A32064
LentiArray Cas9 Lentivirus, 1mL	A32069
LentiArray Lentiviral sgRNA	A32042
LentiArray CRISPR Negative Control Lentivirus, human non-targeting, 100 µL	A32062
LentiArray CRISPR Negative Control Lentivirus, human non-targeting, 1 mL	A32327
LentiArray CRISPR Negative Control Lentivirus, human non-targeting with GFP	A32063
LentiArray CRISPR Positive Control Lentivirus, human HPRT	A32056
LentiArray CRISPR Positive Control Lentivirus, human HPRT with GFP	A32060
*mir*Vana Custom miRNA mimic, 5 nmol	4464068
*mir*Vana miRNA inhibitor, Negative Control #1, 5 nmol	4464076
*mir*Vana Pre-designed miRNA inhibitor, 5 nmol	4464084
*mir*Vana Pre-designed miRNA mimic, 5 nmol	4464066
Silencer Select Custom siRNA, 5 nmol	4390827
Silencer Select Negative Control #1, 5 nmol	4390843
Silencer Select Pre-designed Human siRNA, 5 nmol	4392420
Silencer Select Pre-designed Mouse siRNA, 5 nmol	4390771
Silencer Select Validated siRNA, 5 nmol	4390824
TrueCut Cas9 Protein v2	A36498
TrueGuide sgRNA Negative Control, non-targeting	A35526
TrueGuide sgRNA, custom design	A35534
TrueGuide sgRNA, pre-designed	A35533
Differentiation	
Activin A Recombinant Human Protein	PHC9564
B-27 Plus Neuronal Culture System	A3653401
B-27 Plus Supplement	A3582801
B-27 Supplement (50X) minus insulin	A1895601
B-27 Supplement (50X) minus vitamin A	12587010
B-27 Supplement (50X), serum free	17504044
bFGF Recombinant Human Protein	13256029
CTS Essential 6 Medium**	A4238501

Product	Cat. No.
CultureOne Supplement	A3320201
EGF Recombinant Human Protein	PHG0311
Essential 6 Medium	A1516401
GM-CSF Recombinant Human Protein	PHC2015
Neurobasal Medium	21103049
Neurobasal Plus Medium	A3582901
PSC Cardiomyocyte Differentiation Kit	A2921201
PSC Definitive Endoderm Induction Kit	A3062601
PSC Dopaminergic Neuron Differentiation Kit	A3147701
PSC Neural Induction Medium	A1647801
TNF Recombinant Human Protein	PHC3015
VEGF Recombinant Human Protein	PHC9394
Characterization	
Alexa Fluor 488 CD44 Live Cell Imaging Kit	A25528
Alexa Fluor 488 TRA-1-60 Live Cell Imaging Kit	A25618
Alexa Fluor 555 TRA-1-60 Live Cell Imaging Kit	A24879
Alexa Fluor 594 TRA-1-60 Live Cell Imaging Kit	A24882
Alkaline Phosphatase Live Stain	A14353
c-Myc Antibody	MA1-980
DNMT3b Antibody	49-1028
Human Cardiomyocyte Immunocytochemistry Kit	A25973
Human Neural Stem Cell Immunocytochemistry Kit	A24354
KaryoStat Assay	905403
KaryoStat HD Assay	905404
KLF4 Antibody	710659
LIN28 Antibody	MA1-016
NANOG Antibody	MA1-017
OCT4 Antibody	A13998
Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit	A24881
PrimeView 16 Global Gene Expression Profile Assay	905402
PrimeView Global Gene Expression Profile Assay	905400
TaqMan hPSC Scorecard Panel, 96-well	A15871
TaqMan hPSC Scorecard Panel, Fast 96-well	A15876
TaqMan hPSC Scorecard Panel, 384-well	A15870
TaqMan hPSC Scorecard Kit, 384-well	A15872

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