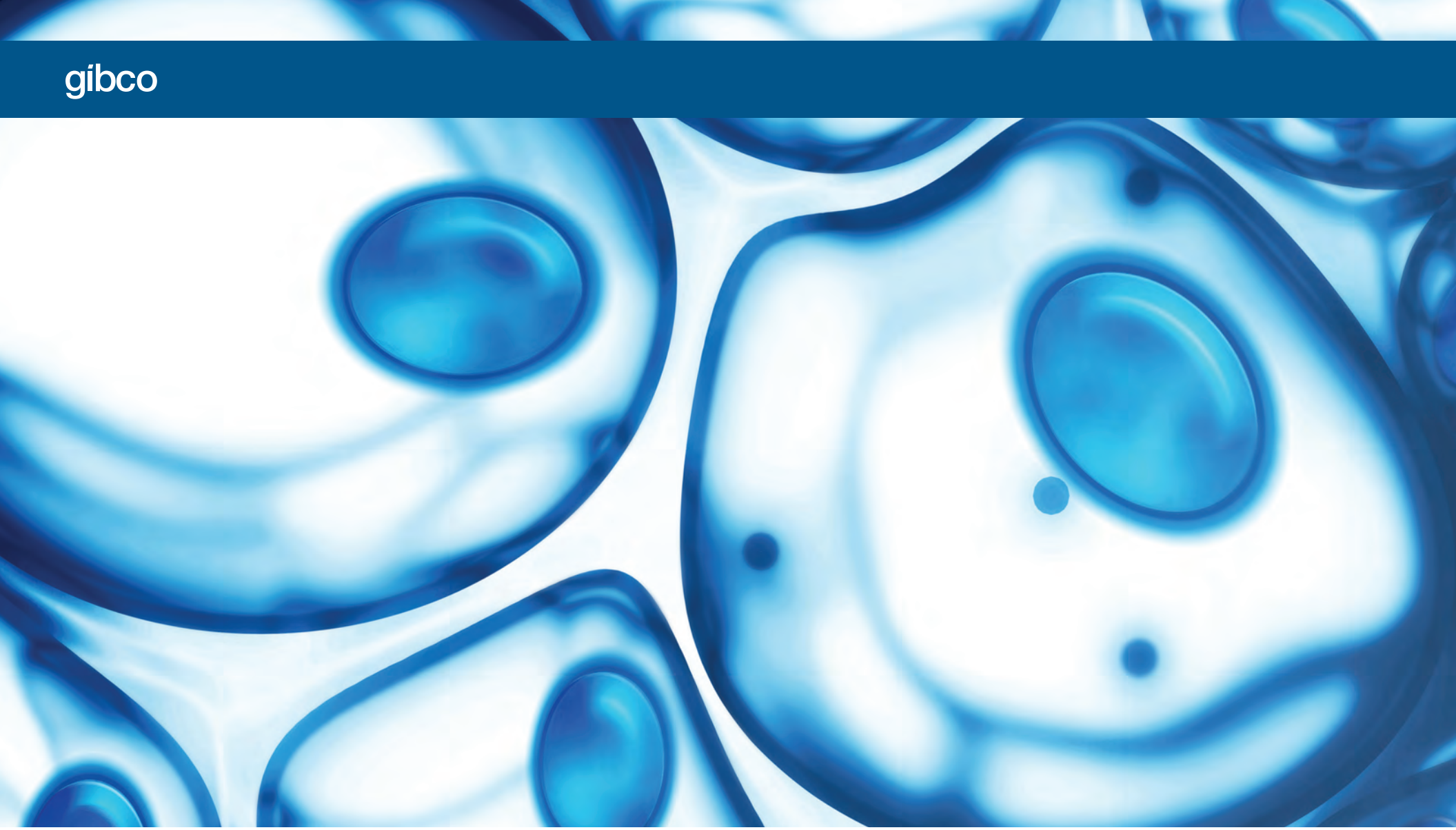
gibco



Pluripotent stem cell product guide

Key products and services for PSC research

ThermoFisher SCIENTIFIC

The pluripotent stem cell workflow

Somatic and progenitor cells

The reprogramming process starts with human cells

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

Media
StemPro-34 SFM

Cell culture plastics
Nunc flasks, dishes, and plates

Reprogram

Reprogram adult cells such as blood cells back to a pluripotent state to become iPSCs

CytoTune-iPS 2.0 Sendai Reprogramming Kit
CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit
Episomal vectors
Epi5 vectors
Lipofectamine 3000 Transfection Reagent
Lipofectamine MessengerMAX Reagent
Nunclon Delta and ECM-coated plates

Culture

iPSC expansion requires careful attention to culture conditions

Culture media
StemFlex Medium
Essential 8 Medium
Essential 8 Flex Medium
CTS Essential 8 Medium
KnockOut Serum Replacement – Multi-Species

Supplements
RevitaCell Supplement

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

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

thermofisher.com/pscculture

Engineer

Engineer your induced pluripotent stem cells to analyze gene and protein expression and study differentiation and disease pathways

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

thermofisher.com/transfection

Gene editing
TrueCut Cas9 Protein v2
TrueGuide Synthetic gRNAs
PerfectMatch TALs

thermofisher.com/genomeedit

Cell culture plastics
Nunclon Delta and ECM-coated plates

Differentiate

Once cells have been reprogrammed, they are then differentiated into specific cell types

Ectoderm
B-27 Plus Neuronal Culture System
CultureOne Supplement
PSC Dopaminergic Neuron Differentiation Kit
PSC Neural Induction Medium

Mesoderm
PSC Cardiomyocyte Differentiation Medium

Endoderm
PSC Definitive Endoderm Induction Medium Kit

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

thermofisher.com/differentiation

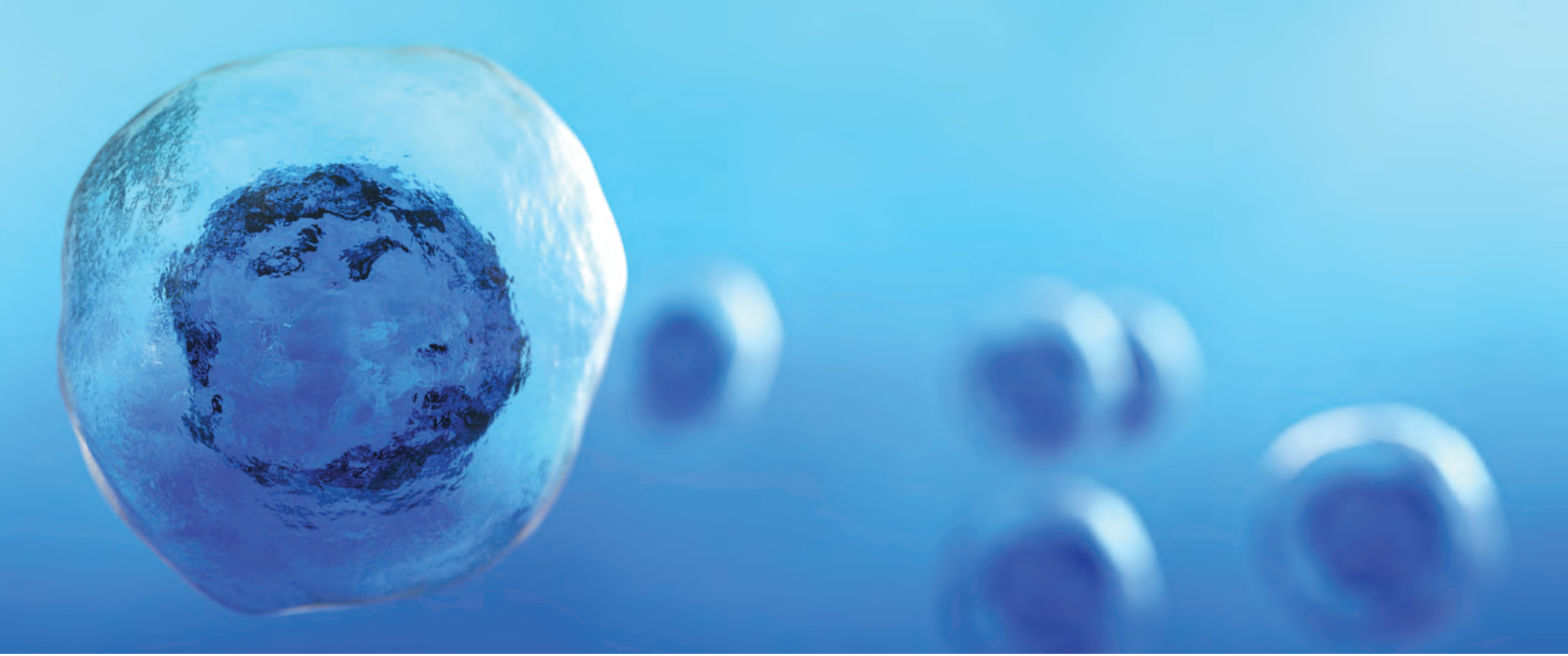
Characterize

Multiple methods from basic to more advanced characterization are required throughout, as validation is critical in iPSC research.

thermofisher.com/detectpsc

CellInsight CX7 High Content Screening (HCS) Platform
Lab-Tek and Lab-Tek II Chamber Slides and Chambered Coverglasses
Nunc Glass Bottom Dishes and Optical Bottom Plates
KaryoStat and KaryoStat HD Assays
TaqMan hPSC Scorecard Panel
Primary and secondary antibodies
PluriTest-compatible PrimeView Global Gene Expression Profile Assays
Attune NxT Flow Cytometer
EVOS cell imaging systems
GeneArt Genomic Cleavage Detection and Selection Kits
Immunocytochemistry and live staining kits

Contents



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. We 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.

Our wide range of products and services allows you to simplify your workflow and provides you with more control, allowing for faster, more efficient systems.

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

Whether the final goal of your experiment is to understand the basic biology of cells or to reprogram the cells to eventually differentiate 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

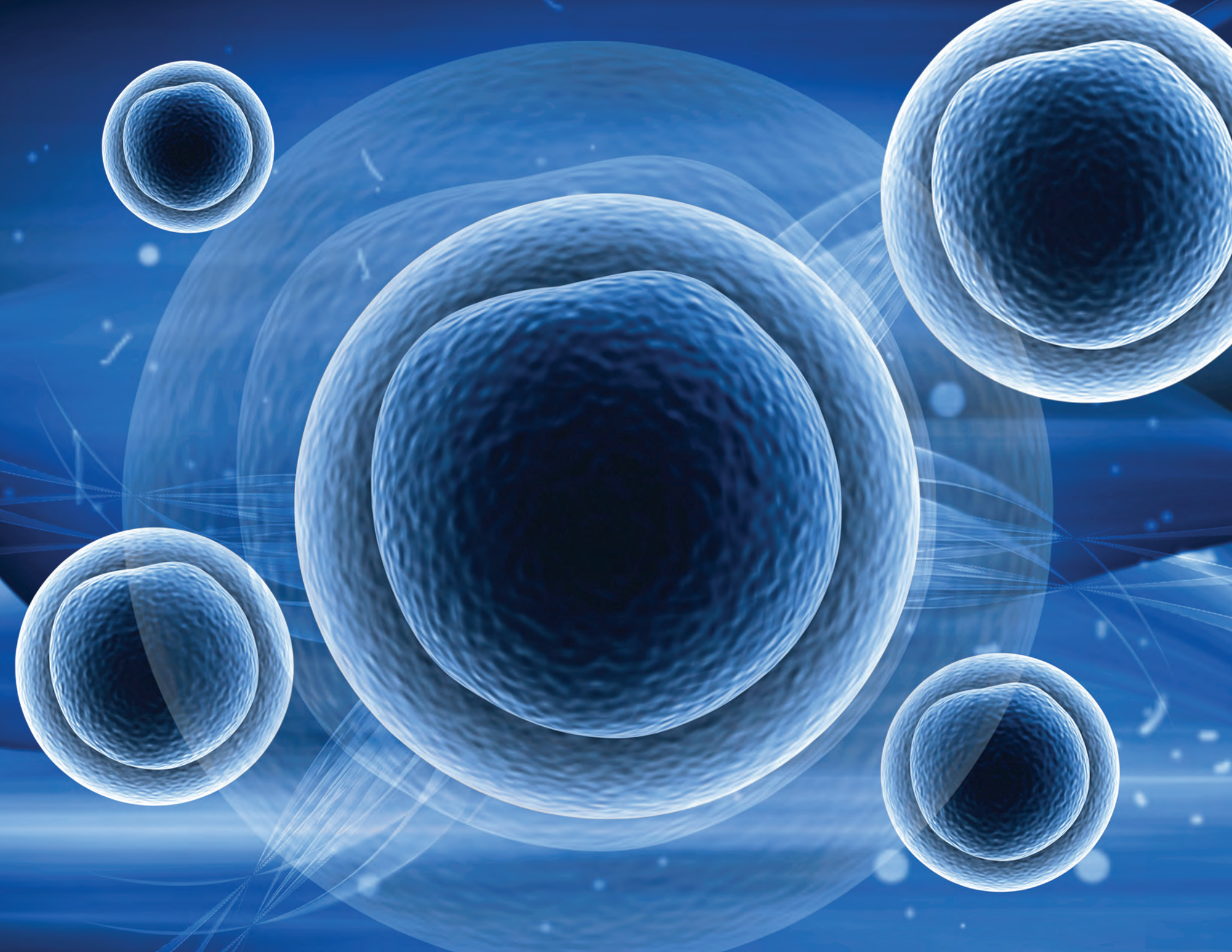
- To request the MSC Sourcebook, a product reference guide supporting your MSC/ADSC workflow, go to **thermofisher.com/mscbook**
- View stem cell protocols for expanding somatic cells at **thermofisher.com/stemcellprotocols**

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	StemPro Neural Stem Cells	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 compliance	Media	Media and cells	Media	Media	Media	Media and cells	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 use on the order of a physician.



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 reprogramming 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.

Go to **thermofisher.com/reprogramming** to find the best solution for your reprogramming experiment.

Support resources

- View cell reprogramming protocols at **thermofisher.com/stemcellprotocols**
- 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%	0.02–1.2%	0.01–0.6%
Genes utilized	Thomson/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	Lipofectamine 3000 Transfection Reagent–based	Transduction	Transduction

* Commercialized in partnership with Cellular Dynamics International.

** Designed by CiRA/Dr. 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 page 52 for all of our stem cell services.

CytoTune-iPS Sendai Reprogramming Kits

Highest success rate among nonintegrating reprogramming technologies

The Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit contains 3 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, up to 1.2% (Figure 1). This polycistronic vector has a different backbone containing temperature-sensitive mutations to polymerase-related genes, which helps to clear the virus faster after reprogramming and causes less cytotoxicity to the cells.

This superior system enables:

- 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

For more information on CytoTune reprogramming, go to **thermofisher.com/cytotune**

Seamless transition to the clinic

Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit

- First off-the-shelf reprogramming system manufactured in accordance with GMP requirements
- Xeno-free workflow for generation of iPSC lines from both fibroblasts and blood for clinical research
- The CTS CytoTune 2.1 kit offers the high-efficiency Sendai delivery of reprogramming factors, and extensive testing and documentation, including an FDA Drug Master File, to support your regulatory submission

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

Human		Nonhuman
Adult and neonatal dermal fibroblasts	Nasal epithelial cells	Chimpanzee peripheral mononuclear cells
Amniotic fluid MSCs	Peripheral blood mononuclear cells (PBMCs)	Macaque dermal fibroblasts
Cardiac fibroblasts	Skeletal myoblasts	Mouse embryonic fibroblasts
$CD34^+$ blood cells	T cells	Rhesus monkey dermal fibroblasts
Conjunctival cells	Umbilical vein epithelial cells	
Dental pulp stem cells	Urine epithelial cells	
Mammary epithelial cells		

For publications citing Sendai virus for iPSC generation, go to **thermofisher.com/sendaipubs**

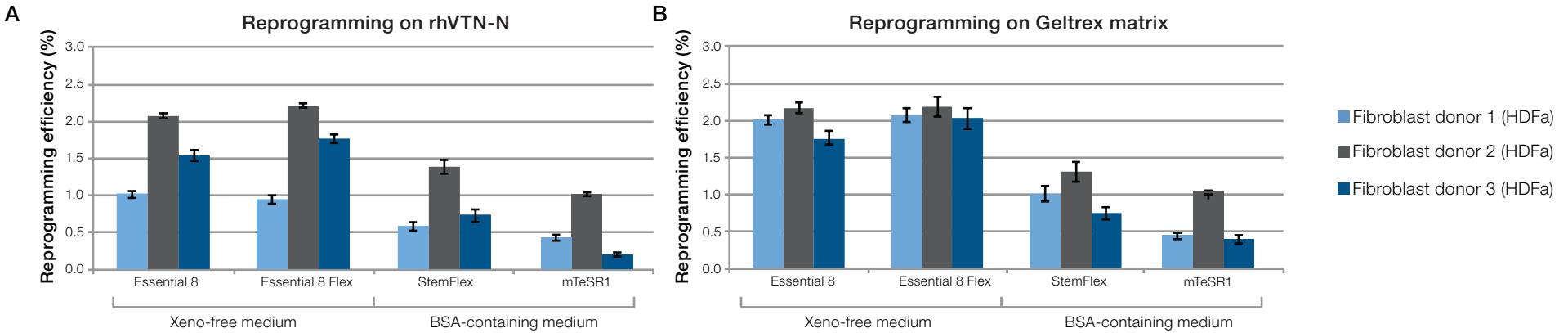


Figure 1. Reprogramming efficiency of human dermal fibroblasts using feeder-free medium conditions on Gibco™ Geltrex™ and rhVTN-N substrates. Fibroblasts from three donors, two adult and one neonatal, were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 7, 50,000 viable cells were 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 mTeSR1™ 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).

Need even better reprogramming efficiency?

Supplement PSC culture media on day 7 of reprogramming with Gibco™ RevitaCell™ Supplement or, alternatively, transfer cells to rhLaminin-521 matrix on day 7 of reprogramming.

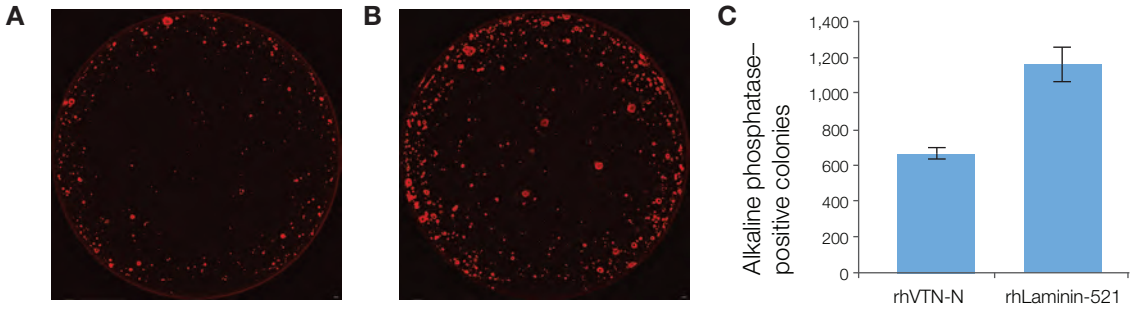


Figure 2. Improvement of feeder-free reprogramming efficiency using alternative matrices or addition of RevitaCell Supplement on day 7 transfer. Feeder-free reprogramming of human dermal neonatal fibroblasts (HDFn) (Cat. No. C0045C) was completed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at a multiplicity of infection (MOI) of 5:5:3. On day 7 posttransduction, reprogrammed fibroblasts were transferred to 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.

Characterization tools

Alkaline Phosphatase Live Stain

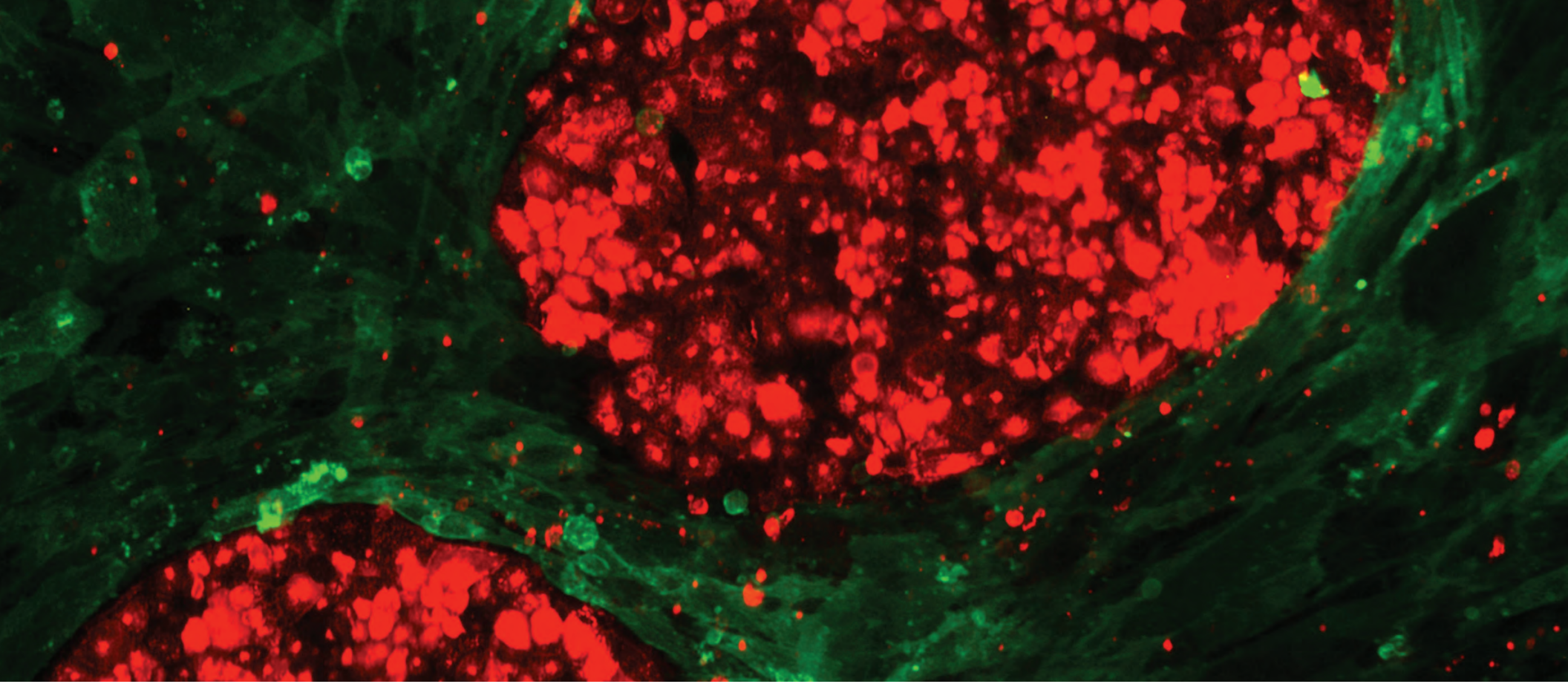
Invitrogen™ Alkaline Phosphatase Live Stain is used for stem cell imaging that allows you to differentially stain PSCs. The dye is a cell-permeant fluorescent substrate for alkaline phosphatase (AP) that is nontoxic to cells, diffusing away over the course of 2 hours.

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 the positive PSC markers and the negative PSC markers are particularly useful.

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



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.

Go to **thermofisher.com/pscculture** to find the right PSC media for your research.

Support resources

- View cell culture protocols at **thermofisher.com/stemcellprotocols**
- Access Essential 8 Medium how-to videos at **thermofisher.com/essential8howto**

Table 4. Media systems for PSC culture.




		Feeder-dependent culture	Feeder-free culture		
Medium		**KnockOut Serum Replacement – Multi-Species**	**StemFlex Medium**	**Essential 8 Medium**	**CTS Essential 8 Medium**
Ideal for		Feeder-based human and mouse 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	Consistent PSC culture and superior reprogramming	Translational or clinical research applications
Defined		Animal-origin components (BSA)	Animal-origin components (BSA)	Xeno-free—no animal-derived components, human derived	Animal origin–free (AOF)
Recommended cell types		Mammalian PSCs	Human PSCs	Human PSCs	Human PSCs
Weekend-free feeding schedule		No	Yes	Yes, Essential 8 Flex Medium	No
Genome editing		Fair	Best	Fair	Fair
Robustness		Good	Best	Fair	Fair
Recommended matrix		Mouse Embryonic Fibroblasts and Attachment Factor (for human and mouse)	Geltrex Matrix rhLaminin-521	Vitronectin (VTN-N) Recombinant Protein rhLaminin-521	CTS Vitronectin (VTN-N) Recombinant Protein rhLaminin-521
Recommended level of dissociation and passaging reagent	**Clump**	Collagenase IV (for human)	Versene Solution	Versene Solution	Versene Solution
	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 wth addition of RevitaCell Supplement recommended during the first 18–24 hours post-passage for improved recovery
	Single cell	TrypLE Express Enzyme (for mouse)	TrypLE Select or Express Enzyme, RevitaCell Supplement helpful but not required during recovery if using rhLaminin-521	TrypLE Select or Express Enzyme with RevitaCell Supplement added to the medium during first 18–24 hours post-passage	CTS TrypLE Select Enzyme with RevitaCell Supplement added to the medium during first 18–24 hours post-passage

Need help growing and banking your iPSC cell line?

Our team of dedicated stem cell scientists can help you create your iPSC banks using the latest Gibco™ media. See page 52 for all of our stem cell services.

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. Many feeder-free stem cell media contain 20 or more components in their formulations (Table 5). While these media may adequately grow and maintain PSCs, they also contain many variables and commonly exhibit lot-to-lot inconsistencies. By removing highly undefined proteins and components (such as BSA and others) 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
- Ideal for clinical or translational research applications
- Modular options to maximize application performance (Table 6)
- No BSA or HSA

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

Table 6. Choose the Essential 8 media system that is best for your application.

Application	Medium	Recommended pairing
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 (including weekend-free)	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 5. Comparison of published PSC medium formulations. Essential 8 Medium makes use of much fewer components to support PSC growth and expansion compared to STEMCELL Technologies' mTeSR1 medium. 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	•	

CTS Essential 8 Medium

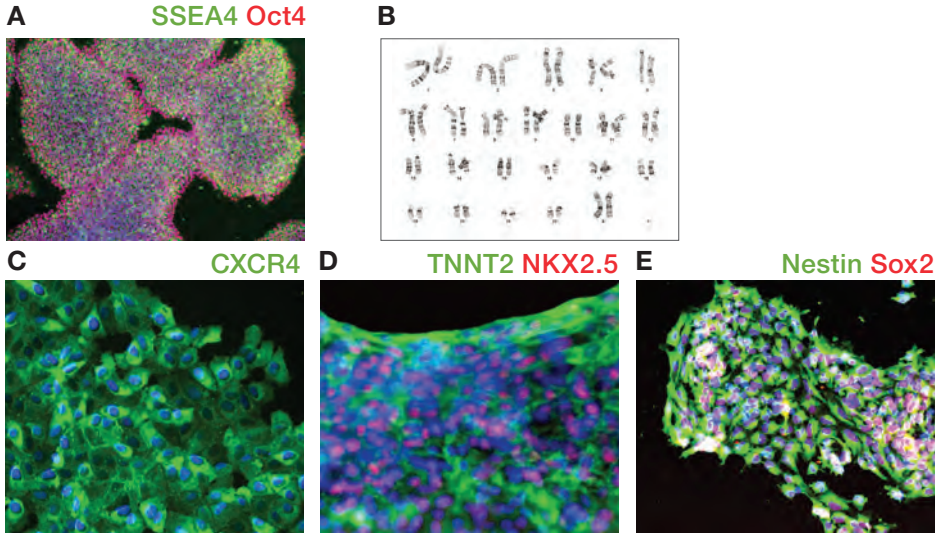
The only globally available animal origin–free hPSC culture medium designed to meet global cell therapy requirements

Based on the widely published Essential 8 Medium, we have developed a Gibco™ Cell Therapy Systems (CTS™)-grade, fully defined human pluripotent stem cell culture medium. CTS Essential 8 Medium offers all of the same benefits of the research-use product, but with fully animal origin–free (AOF) components to support clinical research applications.

Why CTS Essential 8 Medium?

- **Reduces risks**—animal and human origin–free, fully defined, and tested for adventitious agents
- **Facilitates regulatory filings**—cGMP-manufactured and regulatory documentation available, including FDA Drug Master File
- **Provides seamless transition**—same 8-component formulation as research-use Essential 8 Medium, but with AOF components

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



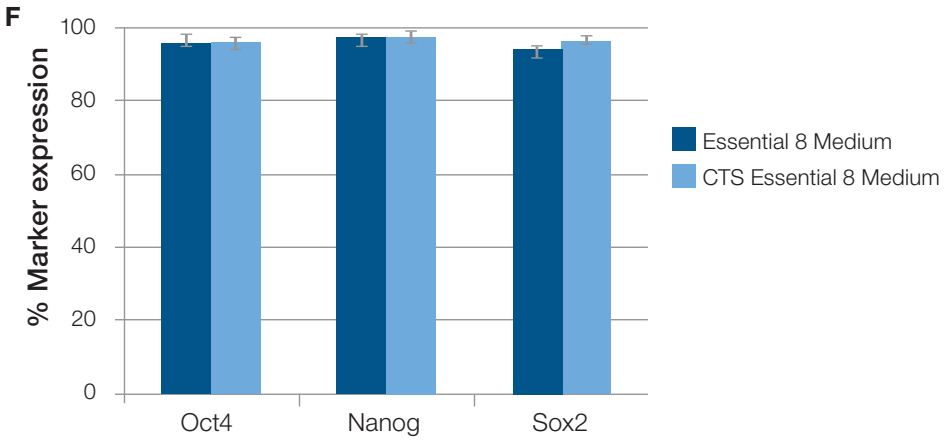


Figure 3. CTS Essential 8 Medium enables long-term PSC culture, trilineage differentiation, and a seamless transition from research-use Essential 8 Medium. PSCs cultured in CTS Essential 8 Medium for >30 passages **(A)** express PSC markers Oct4 and SSEA4 and **(B)** maintain normal 46, XX karyotype. 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.

StemFlex Medium

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

The 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 also 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 4 and 7).

Why StemFlex Medium?

- Superior performance in gene editing, single-cell passaging, and other stressful applications (see page 33)
- Out-of-the-box solution with no optimization or additional reagents required, easy adaptation from other media systems (Figure 5)
- 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**

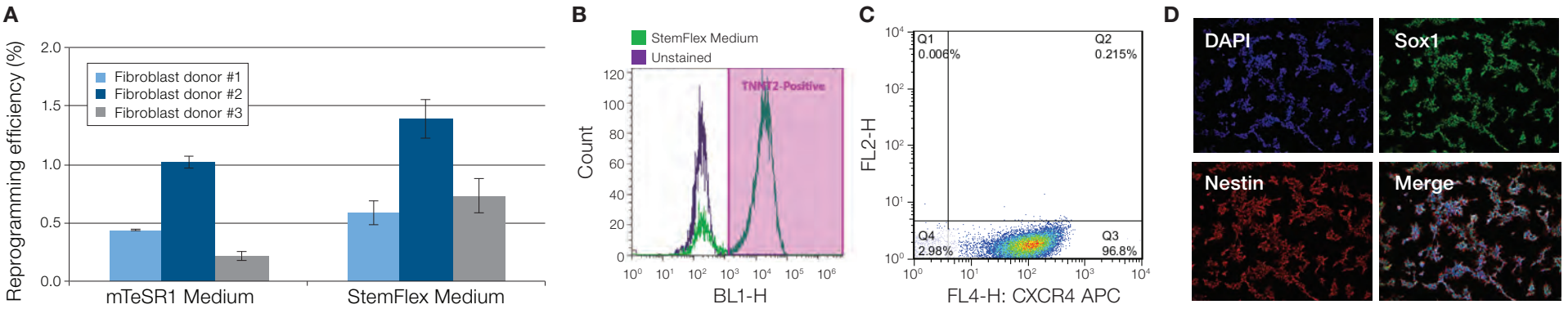


Figure 4. StemFlex Medium provides a robust formulation that can be applied across the entire PSC workflow—from somatic cell reprogramming (A) through downstream differentiation (B–D). When compared to traditional feeder-free media like mTeSR1, StemFlex Medium delivers superior performance across the 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.

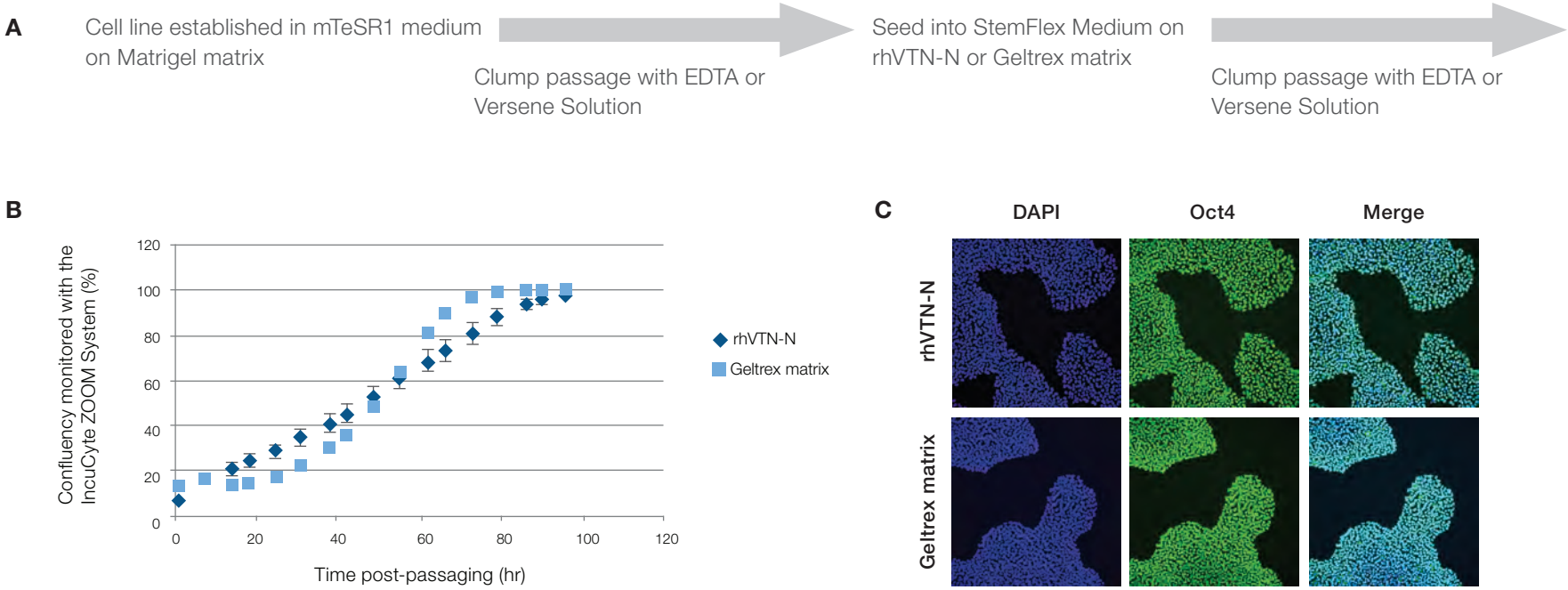


Figure 5. Adaptation of PSCs from mTeSR1 Medium on Matrigel™ matrix to StemFlex Medium on Geltrex matrix or rhVTN-N substrate. (A) Existing PSC lines in mTeSR1 Medium can be easily transitioned to StemFlex Medium following a minimum of two passages for full adaptation. **(B, C)** Cells grow well and exhibit high expression of Oct4 whether on rhVTN-N substrate or Geltrex matrix.

Technical tips

- Allow at least two passages in StemFlex Medium for full adaptation (Figure 5)
- 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. In order to address this weakness in the PSC culture workflow, we have created two unique formulations, Gibco™ 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 (Figure 6 and 7)
- Allow for skipping up to 2 consecutive days for a total of 3 “feeding-free” days in a week (Figure 8)
- Reduce media consumption by up to 30% and thus also reduce costs compared to traditional feeder-free media

To find out more about these media, visit **thermofisher.com/stemflex** and **thermofisher.com/essential8flex**

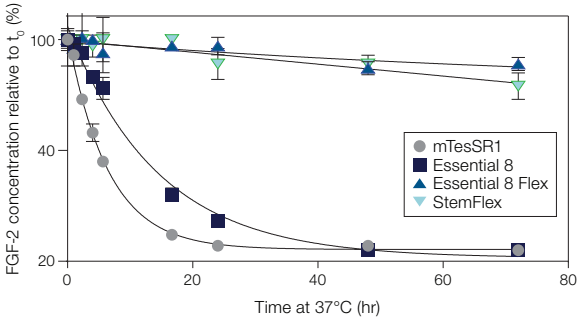


Figure 6. StemFlex and Essential 8 Flex media more consistently maintain pluripotency. Both StemFlex and Essential 8 Flex media provide prolonged FGF-2 stability when incubated at 37°C, 5% CO_2, allowing for flexible feeding schedules, including the weekend-free option—eliminating daily feeding requirements.

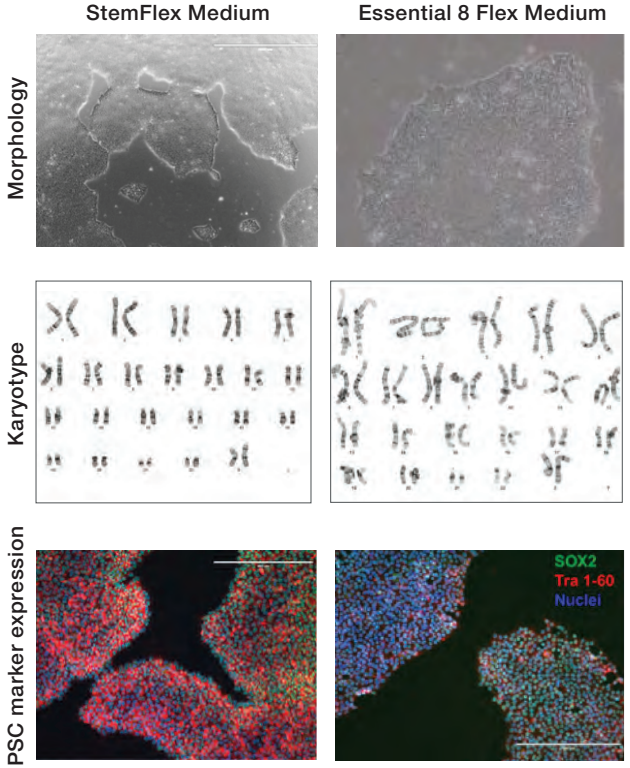


Figure 7. Long-term maintenance of pluripotency in weekend-free feeding schedules. PSCs exhibit normal morphology, karyotype, and expression of pluripotent stem cell markers following 50 passages in StemFlex Medium on Geltrex matrix (left) and in Essential 8 Flex Medium on Gibco™ vitronectin matrix (right).

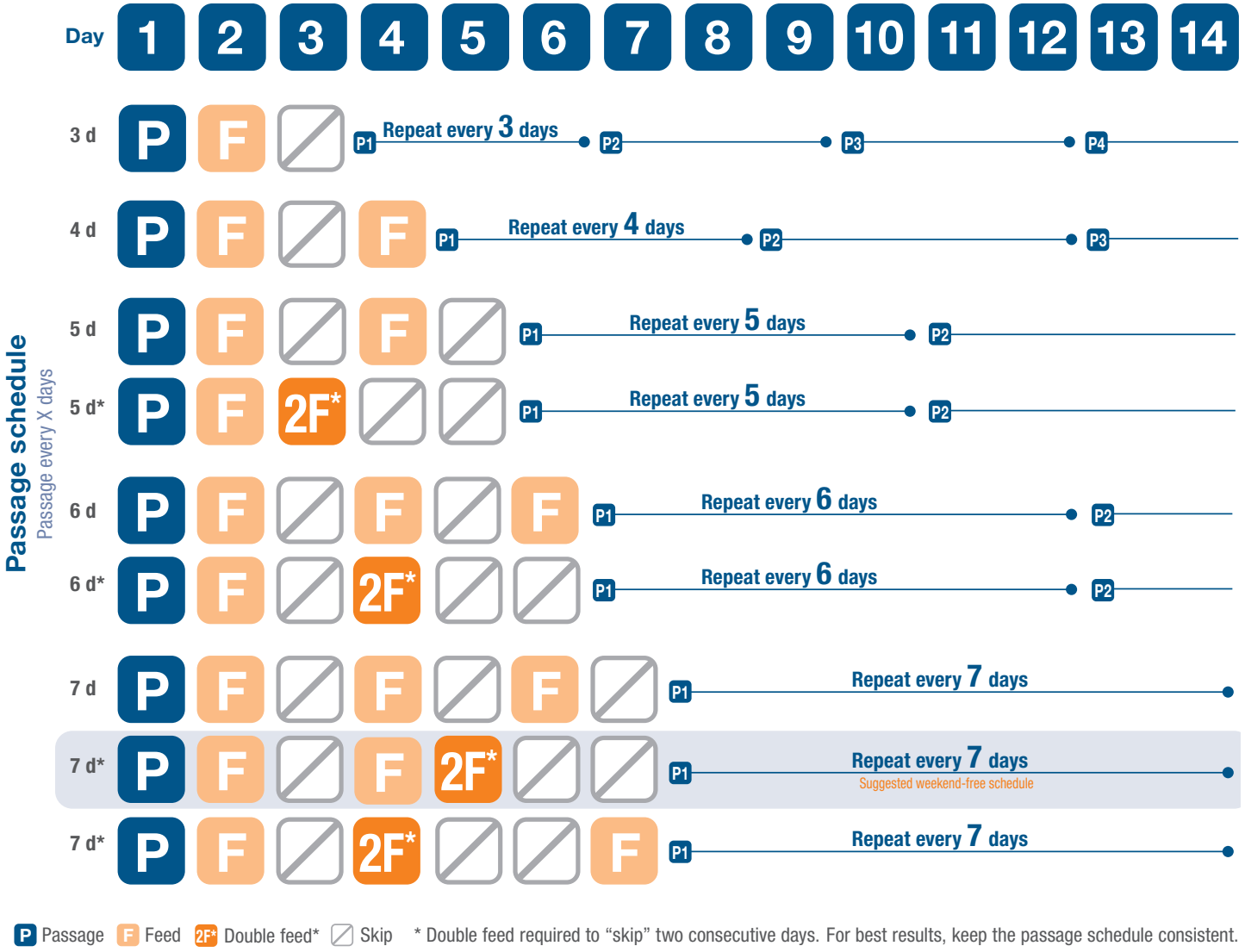


Figure 8. Alternative feed schedules for StemFlex and Essential 8 Flex media.
Note: It is also possible to skip feeding the day after passaging if ROCK inhibitor is not added during passaging.

KnockOut Serum Replacement – Multi-Species

Feeder-dependent culture proven more reliable than FBS

Fetal bovine serum (FBS) is a complex mixture of components that can vary from lot to lot and can be either beneficial or detrimental to PSCs. More defined media have more consistent compositions that reduce the detrimental components and retain the most critical components for PSC maintenance.

Gibco™ KnockOut™ Serum Replacement – Multi-Species (KnockOut SR – Multi-Species) is a more defined, FBS-free culture supplement designed to replace FBS in feeder-based PSC cultures. KnockOut SR – Multi-Species has been proven more reliable than FBS in mouse PSC and human PSC culture (Figures 9 and 10). It offers better maintenance of undifferentiated PSCs at a stable price and stable supply.

Combine it with our broad offering of rigorously tested mouse embryonic fibroblasts (MEFs) manufactured by MTI-GlobalStem.

See the complete set of data and resources at **thermofisher.com/ksrmultispecies**

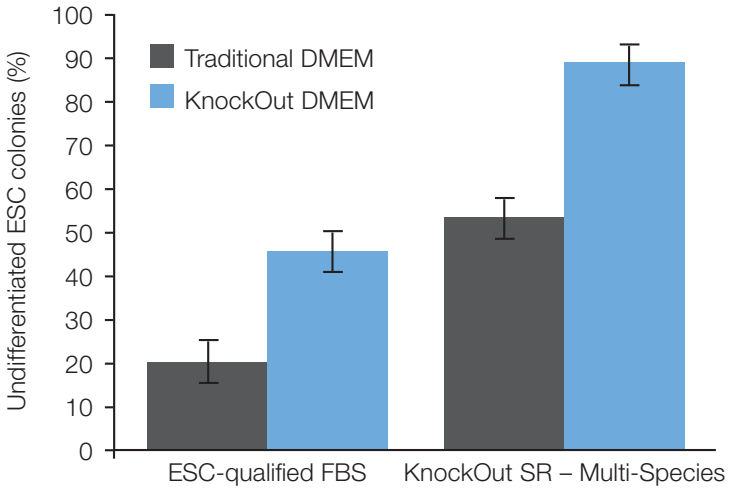


Figure 9. Mouse PSC culture with KnockOut SR – Multi-Species vs. FBS in the absence of leukemia inhibitory factor (LIF). Mouse D3 ESCs were cultured at low density in Gibco™ DMEM or KnockOut™ DMEM supplemented with ESC-qualified FBS or KnockOut SR – Multi-Species. No LIF was used. After 7 days, colonies were fixed and stained for alkaline phosphatase, a marker for undifferentiated ESCs. Undifferentiated colonies were scored based on morphology and staining characteristics.

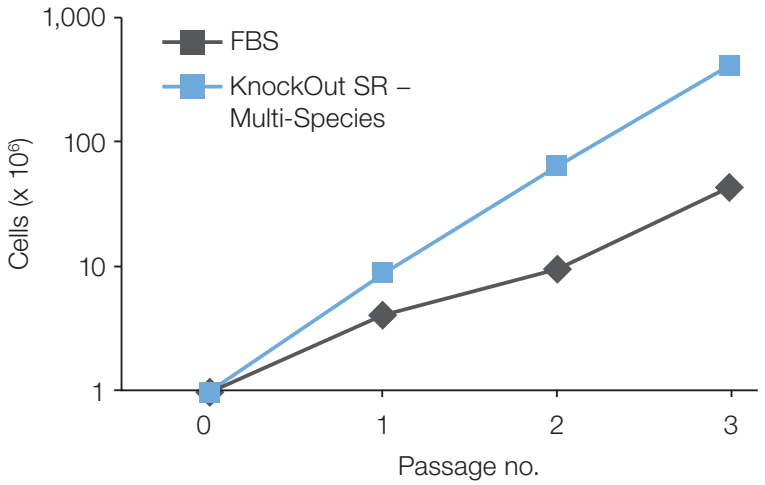


Figure 10. Human PSC growth in KnockOut SR – Multi-Species vs. FBS. H9 human ESCs were cultured on mouse embryonic fibroblasts (MEFs) with 20% ESC-qualified FBS or 20% KnockOut SR – Multi-Species. The mean viable cell numbers were plotted as growth curves for the two types of media. Proliferation of human ESCs was significantly higher in KnockOut SR – Multi-Species 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.

For more efficient recovery, choose RevitaCell Supplement, which has been optimized for use with PSCs as a post-thaw recovery solution to improve cell viability.

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	No*
CTS product available	NA	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.

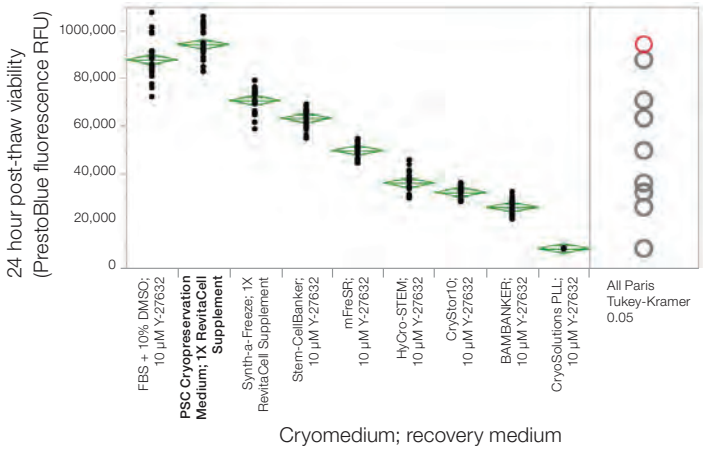


Figure 11. The PSC Cryopreservation Kit provides optimum 24 hour post-thaw cell survival. H9 ESCs cultured in Essential 8 Medium were cryopreserved in various cryopreservation media and subsequently recovered in Essential 8 Medium supplemented with either 10 µM Y-27632 or 1X RevitaCell Supplement. Cell 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.

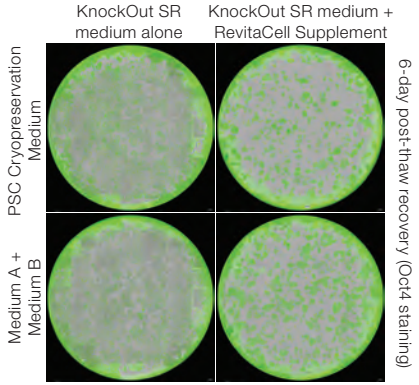


Figure 12. The PSC Cryopreservation Kit also shows utility for cryopreservation of feeder-dependent iPSCs. Feeder-dependent episomal iPSCs were cryopreserved in PSC Cryopreservation Medium or traditionally recommended Medium A + B. PSCs were then recovered in KnockOut SR medium–based feeder-dependent 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.

Characterization tools for pluripotent stem cells

Verifying the quality of your pluripotent stem cells (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. From the Applied Biosystems™ PluriTest-compatible PrimeView™ Global Gene Expression Profile Assays, which provides quick verification of pluripotency, to Applied Biosystems™ TaqMan® hPSC Scorecard Panel, which confirms trilineage differentiation potential, we have the tools you need to characterize with lines with confidence.

Go to **thermofisher.com/characterization** to find the right assay for your research.

Table 8. 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**	**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 donwloadable 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?

We have a dedicated team of stem cell scientists to help you achieve your project goals.

See page 52 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 qPCR 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

Go to **thermofisher.com/scorecard** to find out more about this innovative technology.

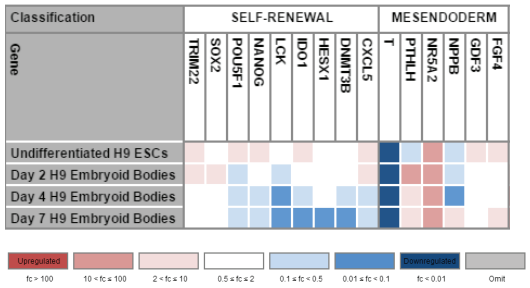


Figure 13. Gene expression results for self-renewal and germ layer markers are summarized in an easy-to-read format.

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
- Free and simple-to-use cloud-based analysis tool

Go to **thermofisher.com/primeview** to find out more about pluripotency verification.

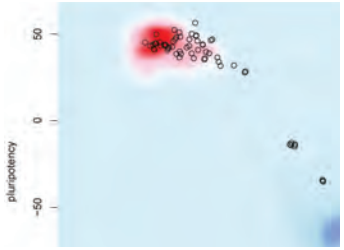


Figure 14. The pluripotency plot is an output of the PluriTest™ Online Analysis Tool and is a visual representation of the pluripotent and nonpluripotent samples in the analysis. The red and blue background hint at the empirical distribution of the pluripotent (red) and nonpluripotent samples (blue) in the reference data set.

KaryoStat Assays

Verify genomic stability

The Applied Biosystems™ KaryoStat™ and KaryoStat™ HD Assays 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

Go to **thermofisher.com/karyostat** to find out more about our G-banding karyotyping alternative.

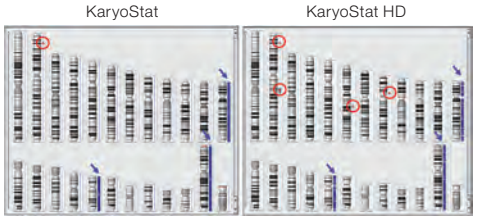
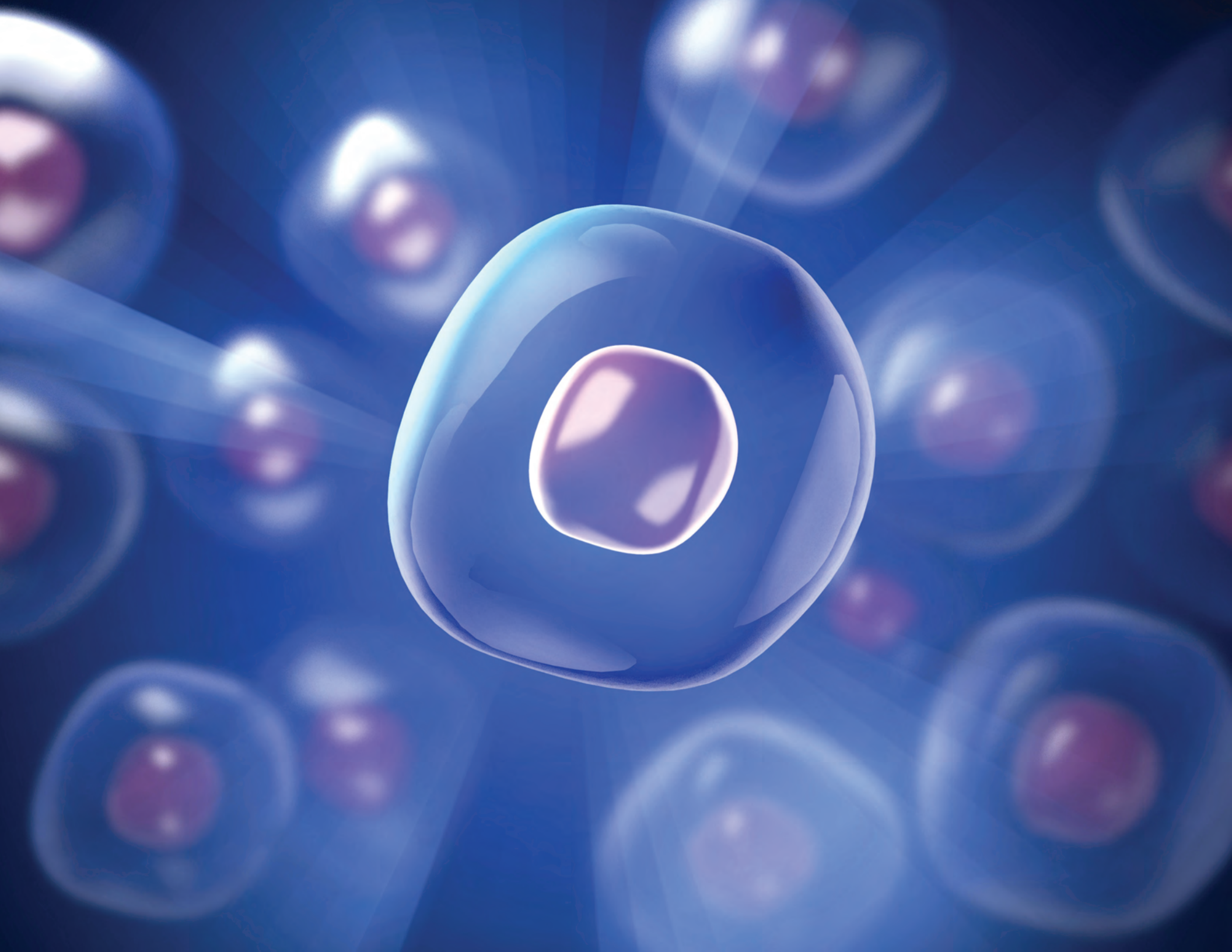


Figure 15. The KaryoStat Assay (left) and KaryoStat HD Assay (right) detect trisomy for chromosomes 12, 17, and X in BG01V, a human embryonic stem cell line with abnormal karyotype. In addition, both assays detect a loss on chromosome 2 that was not detected by G-banding karyotyping.



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**
- Download your copy of our transfection handbook at **thermofisher.com/transfectionhandbook**

Table 9. Transfection selection guide for stem cells. Recommended payloads by transfection method, and transfection efficiency by cell type, are shown. Higher numbers of blocks represent higher efficiency.

Transfection method	Recommended payloads				Transfection efficiency by cell type			
	DNA	mRNA	RNP (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

Need help transfecting your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals.
See page 52 for all of our stem cell services.

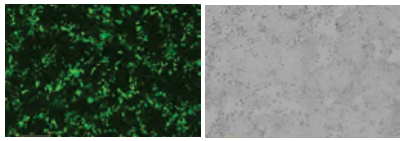
Lipofectamine Stem Transfection Reagent

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.

A iPSCs

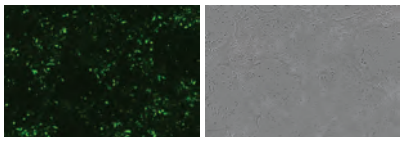
Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well



iPSCs, GFP plasmid
Transfection efficiency: 75%

B ESCs

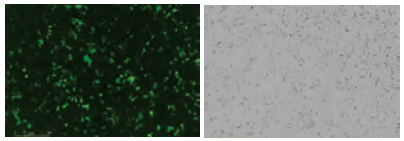
Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 2 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	100,000 cells/well



H9 ESCs, GFP plasmid
Transfection efficiency: 83%

C iPSC-derived NSCs

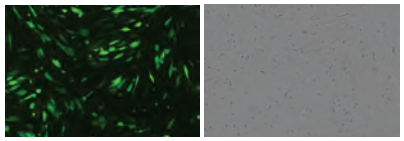
Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	StemPro NSC SFM
Extracellular matrix	Geltrex matrix
Cell density	75,000 cells/well



NSCs, GFP plasmid
Transfection efficiency: 60%

D MSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	MesenPRO RS Medium
Extracellular matrix	CTS CELLstart Substrate
Cell density	25,000 cells/well



Adipose-derived MSCs,
GFP plasmid
Transfection efficiency: 47%

Figure 16. High-efficiency DNA transfection with Lipofectamine Stem reagent in human stem cells.

Lipofectamine Stem reagent is designed to provide you with:

- **Superior efficiency**—achieve up to 80% transfection efficiency in PSCs and NSCs and up to 45% in MSCs (Figure 16)
- **Versatility**—co-delivers DNA (up to 11 kb), RNA, and Cas9 protein complexes; continues cell proliferation without inducing differentiation (Figures 17 and 18)
- **Flexibility**—transfects adherent and suspension cells, offering a simple alternative to electroporation

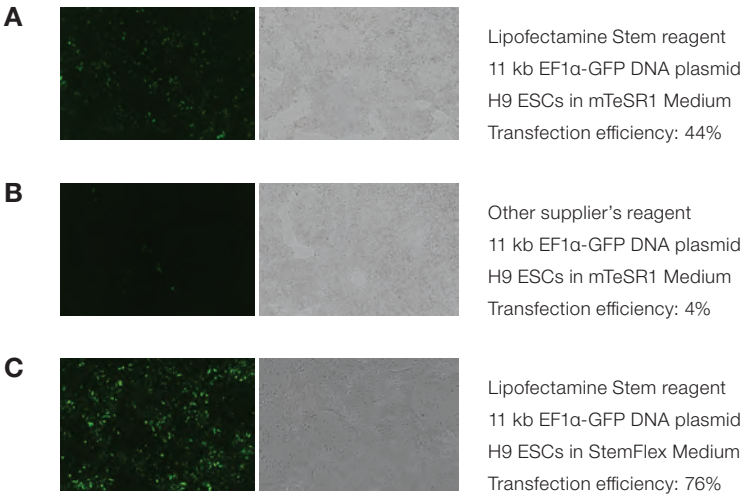
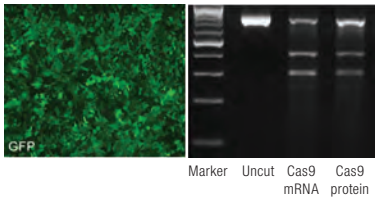


Figure 17. Delivery of a large DNA construct, with significantly higher transfection efficiency than with a leading supplier's reagent. H9 ESCs were transfected with a large 11 kb DNA plasmid using **(A)** Lipofectamine Stem reagent or **(B)** a leading supplier's transfection reagent, and observed 24 hr posttransfection. **(C)** By optimizing culture conditions for transfection, efficiency was nearly doubled.

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
Cas9 protein	500 ng Cas9 protein/125 ng gRNA + 50 ng GFP mRNA
Cas9 mRNA	500 ng Cas9 mRNA/250 ng gRNA + 50 ng mRNA
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well



Transfection efficiency: 90%
Cas9 mRNA: 64% indel
Cas9 protein: 44% indel

Figure 18. Transfection with Lipofectamine Stem reagent supports high-efficiency gene editing in stem cells. (Left) Human iPSCs were cotransfected with Cas9 mRNA, gRNA, and GFP mRNA (not shown), or Cas9 RNP targeting the *EMX1* gene and GFP mRNA. **(Right)** Genomic PCR products of iPSCs were analyzed by a T7 endonuclease I assay (T7 endo) to detect Cas9 cleavage of the *EMX1* gene.

Lipofectamine 3000 Transfection Reagent

Achieve over 60% transfection efficiency in stem cells for just pennies per reaction

Invitrogen™ Lipofectamine™ 3000 Transfection Reagent was developed as a broad-spectrum transfection reagent that achieves efficient, cost-effective nucleic acid delivery across cell types including stem cells. Compared to electroporation, it minimizes the stress on cells caused by electroporation, simplifies the reprogramming workflow, and enables advanced gene editing technologies.

Lipofectamine 3000 reagent is designed to provide you with:

- **High efficiency**—up to 10-fold higher efficiency into the broadest spectrum of difficult-to-transfect cells (Figure 19)
- **Low toxicity**—gentle on cells for improved viability
- **Best value**—just pennies per reaction for superior transfection results*

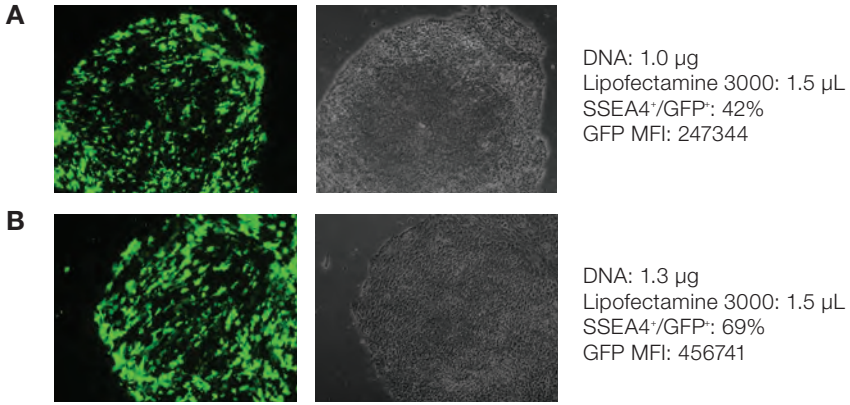


Figure 19. Transfection of stem cells. (A) H9 ESCs or **(B)** iPSCs were transfected using Lipofectamine 3000 reagent. Cells were stained for pluripotency with an SSEA4 antibody, visualized by fluorescence microscopy, and processed using flow cytometry to determine transfection efficiency and SSEA4+ cells.

* In USD, based on a 96-well plate comparison.

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.

Lipofectamine MessengerMAX reagent offers:

- 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

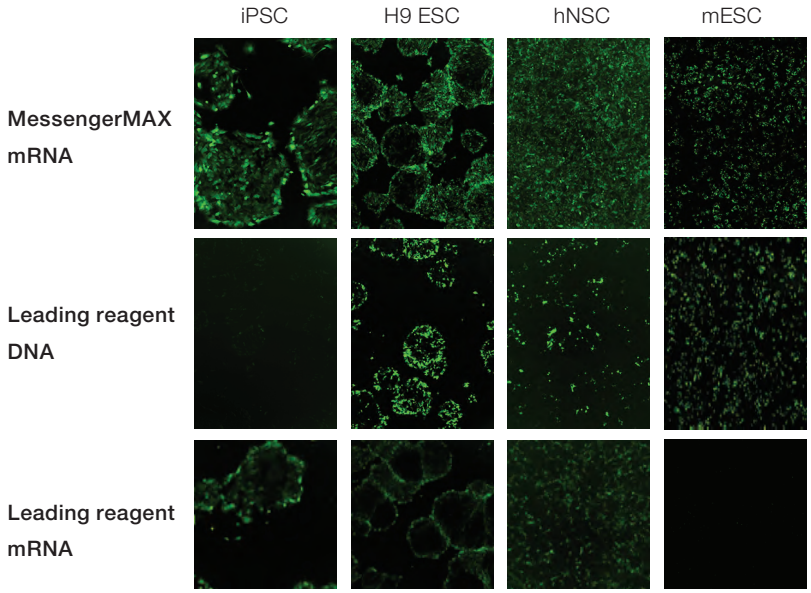


Figure 20. Lipofectamine MessengerMAX reagent outperforms leading DNA delivery reagent and leading mRNA delivery reagent in various stem cells (Gibco iPSCs, H9 ESCs, mESCs, and hNSCs). Lipofectamine MessengerMAX and the leading mRNA delivery reagent were used to deliver GFP mRNA (250 ng/well) in a 48-well 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 difficult-to-transfect cells.

- **Superior results**—up to 90% transfection efficiency and 85% cleavage efficiency in stem cells
- **Gentle**—adjustable parameters enable more gentle transfection and higher viability than other electroporation instruments
- **Customizable**—preprogrammed 24-well optimization protocols and open platform for additional protocols
- **Compatible**—use with StemFlex Medium during genome editing via electroporation

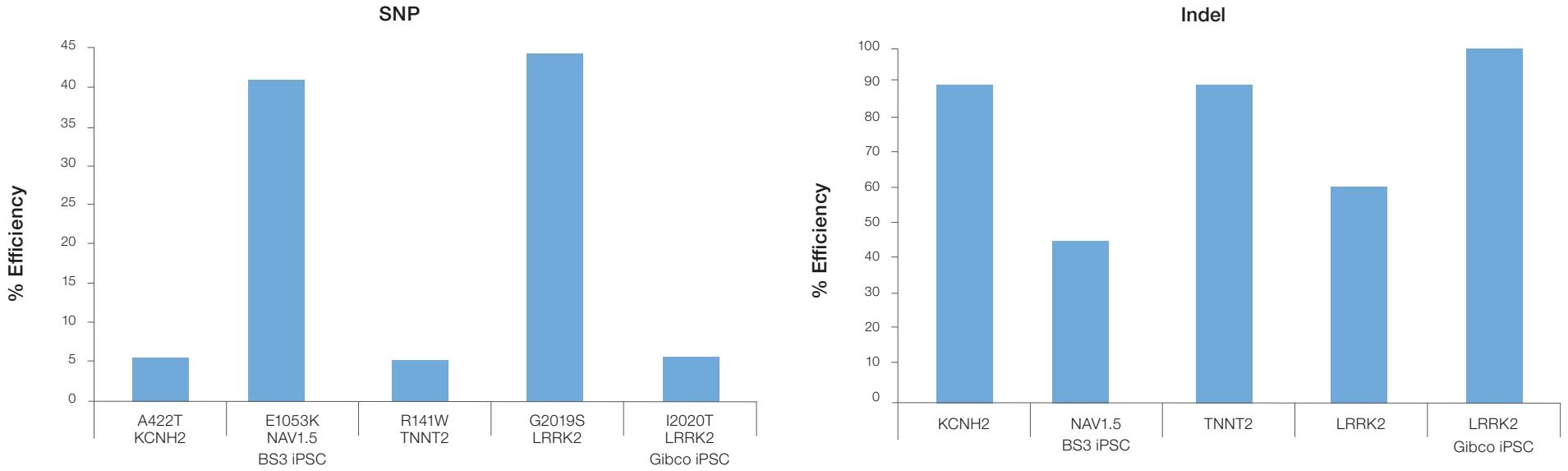
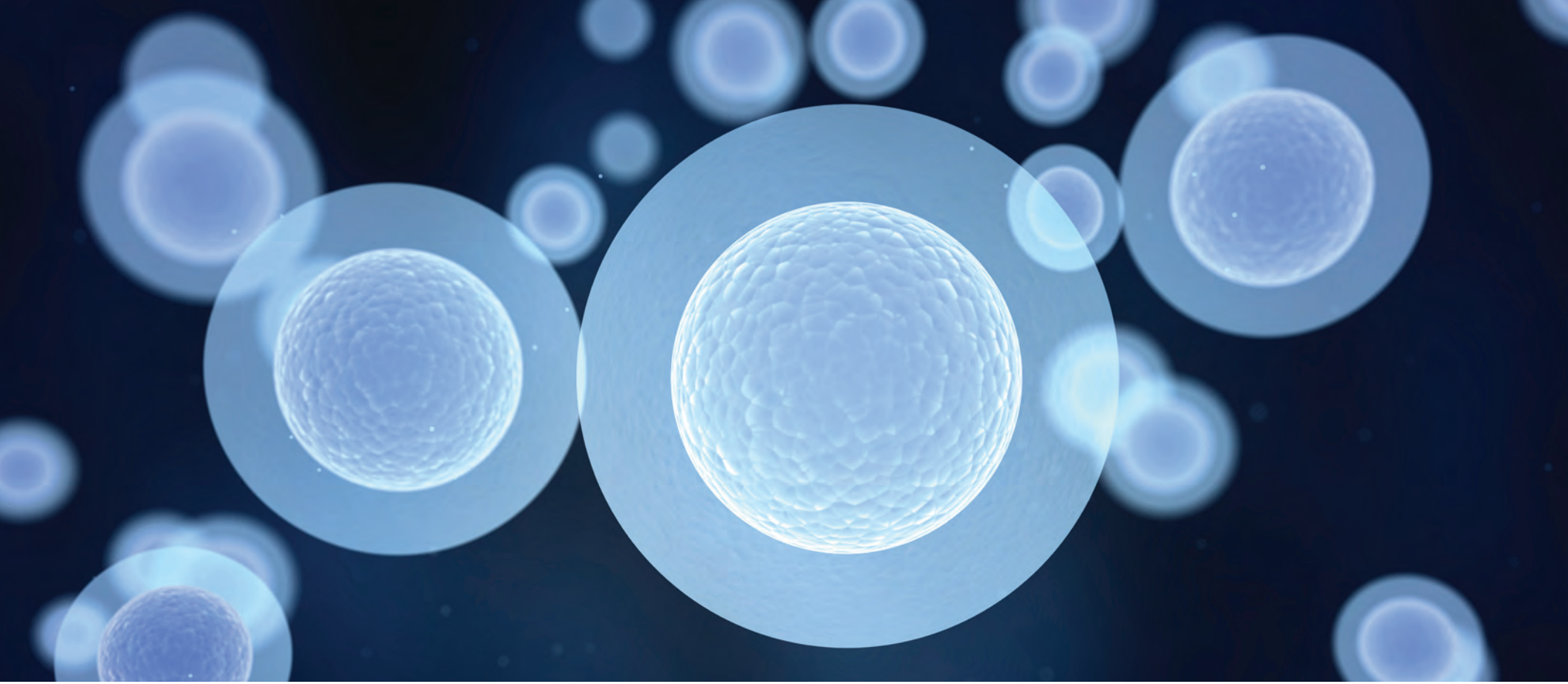


Figure 21. Delivery of Cas9 protein and gRNAs via Neon electroporation allows for efficient genome editing in hiPSC lines across multiple targets. Both NHEJ- and HDR-mediated genome editing can be achieved efficiently, although they target dependently.



Genome editing

Genome editing technologies, such as CRISPR and TAL effector nucleases (TALEN), 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**
- Join our new hands-on CRISPR workshop; find out more at **thermofisher.com/CRISPRworkshop**
- Download the Genome Editing Resource Guide **thermofisher.com/genomeeditresourceguide**

Table 10. Genome editing product overview.

	Single-gene analysis		High-throughput screening	
End goal	Permanent gene knockout or knock-in	Permanent gene knockout, knock-in, or downregulation, gene activation	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 • 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	NA	PAM site (NGG)
Ideal products for PSC	TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNAs	GeneArt TAL effectors	Silencer Select siRNA Libraries	LentiArray CRISPR Libraries or Custom LentiPool CRISPR Libraries
Format	NA	NA	Array or pooled	Array or pooled

TrueCut Cas9 Protein v2

Next-generation Cas9 protein designed to deliver maximum editing efficiency

Introducing Invitrogen™ TrueCut™ Cas9 Protein v2, a wild type Cas9 in protein form designed to deliver consistently higher editing efficiency across a range of gene targets and cell types.

Why TrueCut Cas9 Protein v2?

- Consistently high editing efficiency in all tested cell lines, including standard, immune, primary, and stem cells, with up to 2x higher editing efficiency in difficult targets compared to products from other suppliers
- Manufactured under strict ISO 13485–certified facilities
- Validated protocols for a large number of cell types help you achieve success faster—access these protocols at **thermofisher.com/crisprprotocols**

See the data at **thermofisher.com/crisprprotein**

Need help engineering your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals.
See page 52 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 mouse PSCs, we offer a protocol using KnockOut Serum Replacement – Multi-Species. 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.

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

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.

For custom services and cell lines, visit **thermofisher.com/celllineservice**

Table 11. Available CRISPR-Cas9 delivery formats.

	Cas9 nuclease				CRISPR gRNA			
Formats available	Cas9 protein	Cas9 mRNA	Cas9 lentivirus	Cas9 plasmid	Custom ready-to-transfect gRNA	Catalog ready-to-use gRNA	Design your own gRNA using our CRISPR Design Tool	Catalog packaged as ready-to-use lentivirus gRNA
Editing product	**Award-winning*** TrueCut Cas9 Protein v2	GeneArt CRISPR Nuclease mRNA	LentiArray Cas9 lentivirus	GeneArt CRISPR Nuclease Vector	Invitrogen gRNA custom service	TrueGuide Purified sgRNA	GeneArt CRISPR Search and Design Tool and GeneArt Precision gRNA Synthesis Kit	**Award-winning*** LentiArray Lentiviral sgRNA
Recommended delivery product	Lipofectamine Stem reagent or Neon Transfection System	Lipofectamine Stem reagent or Neon Transfection System	Lipofectamine 3000 reagent or Neon Transfection System	Lipofectamine Stem reagent or Neon Transfection System	NA, delivery method determined by Cas9 nuclease format and cell type			

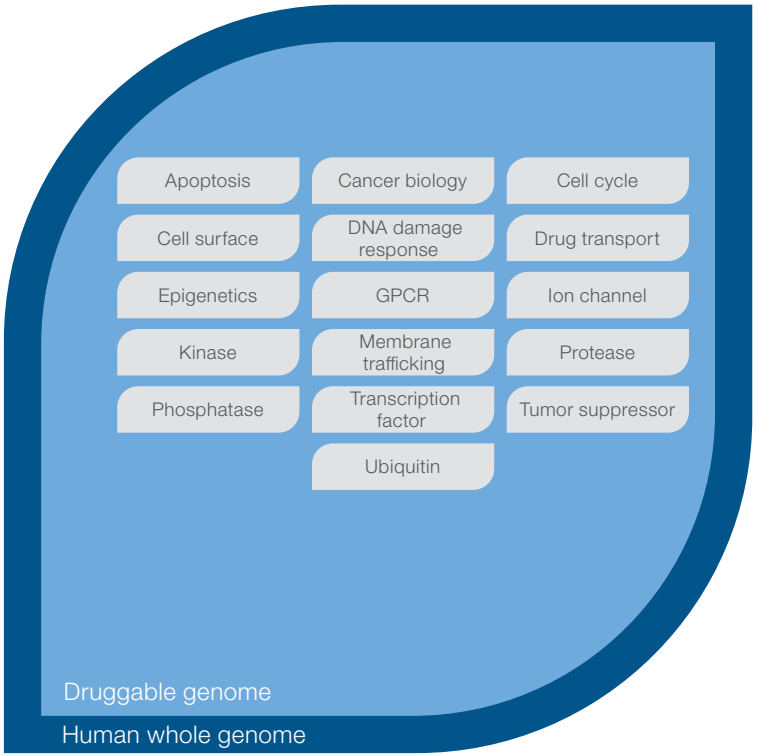
* Awarded Top 10 Innovations in 2017 by *The Scientist* Magazine.

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 next-generation 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 our award-winning Invitrogen™ LentiArray™ libraries. These arrayed CRISPR libraries are designed to provide you with flexible systems that can be adapted to your needs and help drive new discoveries.

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



Human episomal Cas9 iPSC cell line

To create a more robust platform for iPSC genome editing, we stably integrated the Cas9 protein into the Gibco™ Human Episomal iPSC Line.

When used in combination with CRISPR technologies, this new 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 cell line, submit an inquiry at **thermofisher.com/askdiscovery**

Genome editing in stem cells workflow

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 gene editing workflows 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

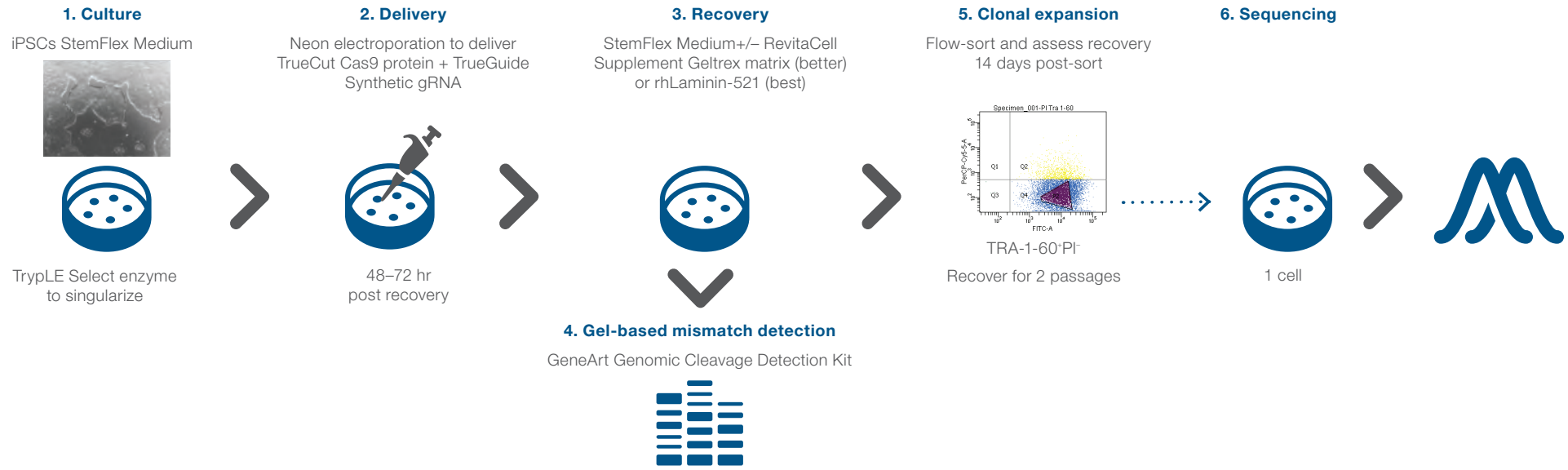


Figure 22. Standard gene editing workflow using CRISPR-Cas9 technology. Following the expansion of hPSCs with the StemFlex Medium and Geltrex matrix system, cells are singularized and electroporated using the Neon system to introduce precomplexed Cas9 protein and control gRNA. Cells recover in StemFlex 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.

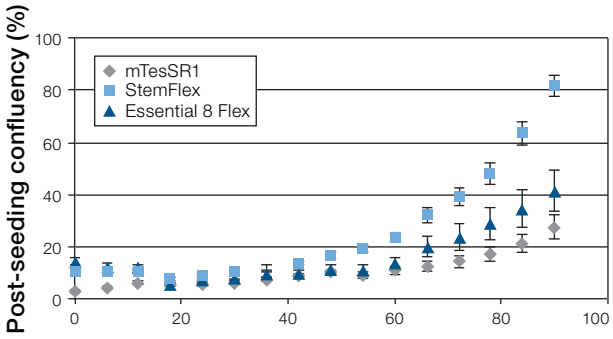


Figure 23. Recovery after singularization and electroporation with Cas9 protein and guide RNA. Cells were seeded at 100,000 viable cells/well of a 24-well plate and allowed to recover in different media. Data shown was generated with cells recovered on a Geltrex matrix.

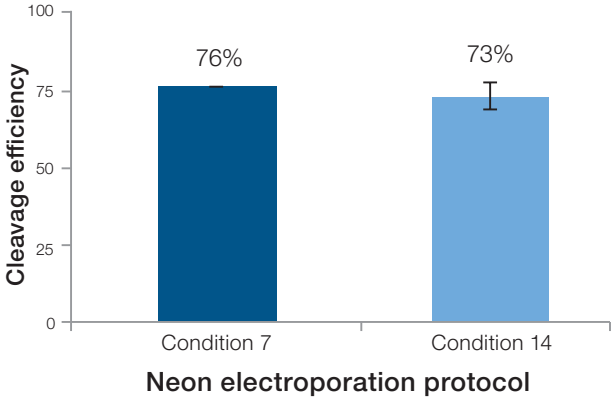


Figure 24. Cleavage efficiency of cultures grown in StemFlex Medium ~72 hours after electroporation with Cas9–gRNA complexes targeting the *HPRT* gene. Condition 7 is 1,200 V, 30 ms pulse width, 1 pulse number, whereas condition 14 is 1,200 V, 20 ms pulse width, 2 pulse number.

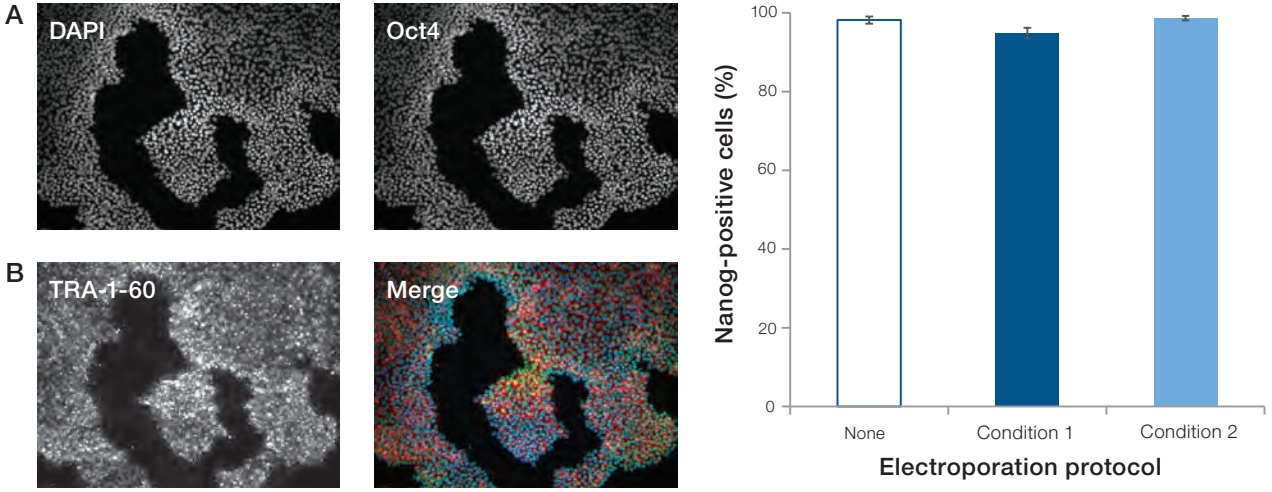


Figure 25. Maintenance of pluripotency of iPSCs cultured in StemFlex Medium after electroporation and recovery. Cultures transfected with Cas9–gRNA complexes targeting the *HPRT* gene were assessed by **(A)** qualitative immunocytochemistry of Oct4 and TRA-1-60 expression and **(B)** quantitative assessment of Nanog expression via flow cytometric analysis.

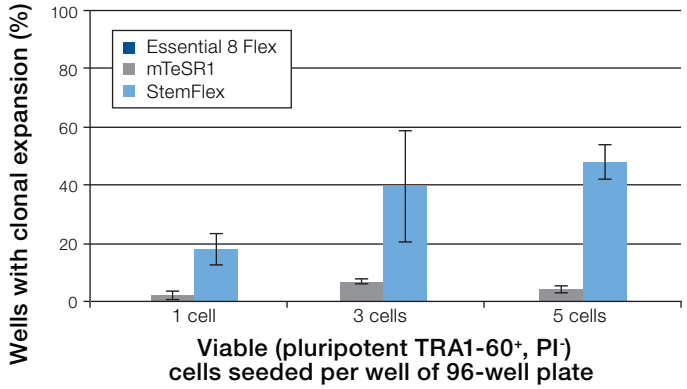


Figure 26. Comparison of cell recovery following flow sorting. Cells were evaluated in the three different media without the need for a ROCK inhibitor and plated at 1, 3, or 5 cells per well on rhLaminin-521. These data demonstrate that StemFlex Medium is the only system that enables significant clonal expansion only when a single cell is plated per well, even in the absence of RevitaCell (or a ROCK inhibitor). Note that the addition of RevitaCell Supplement further boosts cell recovery.

Lipid-based transfection workflow

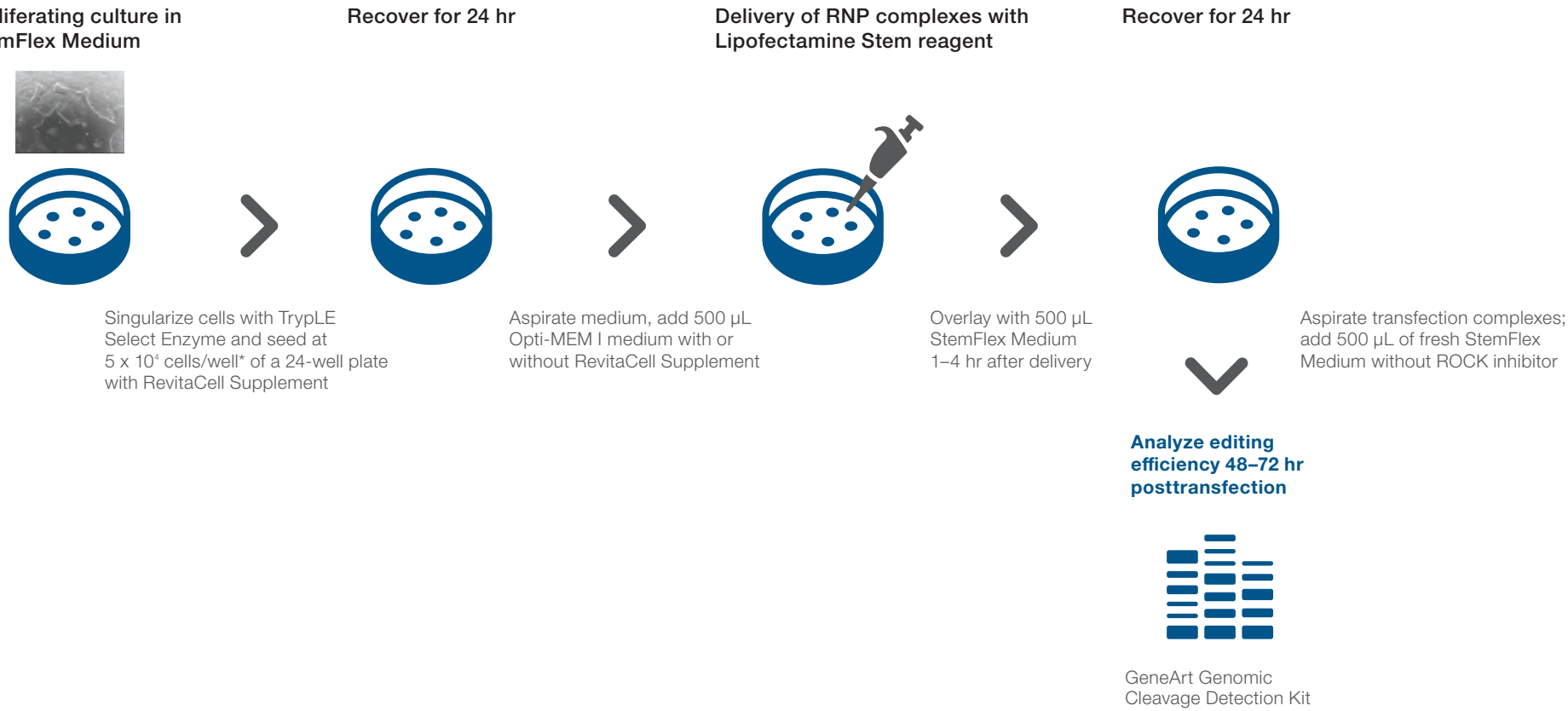


Figure 27. Transfection workflow for delivery of Cas9–gRNA complexes to PSCs cultured in StemFlex Medium using Lipofectamine Stem reagent.

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

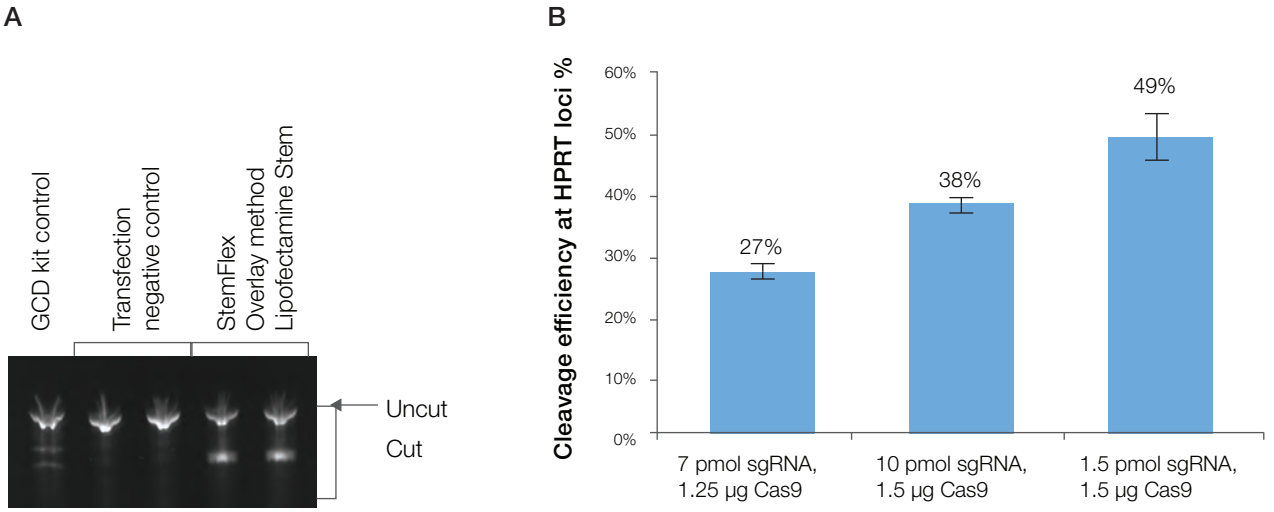


Figure 28. Assessment of indel formation following delivery of Cas9 RNP complex via Lipofectamine Stem reagent. PSCs adapted to StemFlex Medium were single cell–passaged using Gibco™ TrypLE™ Select enzyme and seeded in StemFlex with 1X RevitaCell Supplement at 50,000 viable cells/cm^2 into a 24-well 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-hour 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 loci 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.

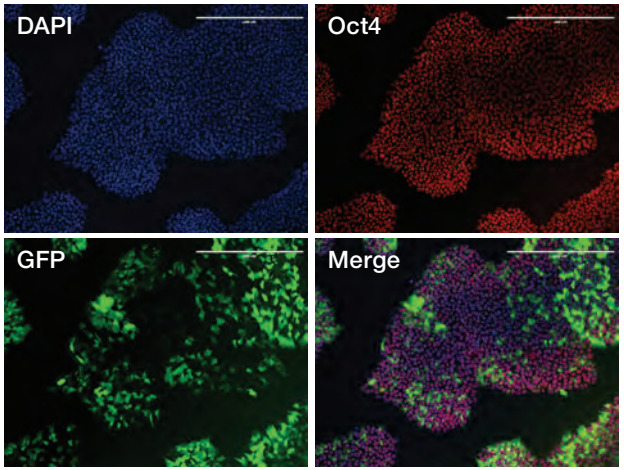
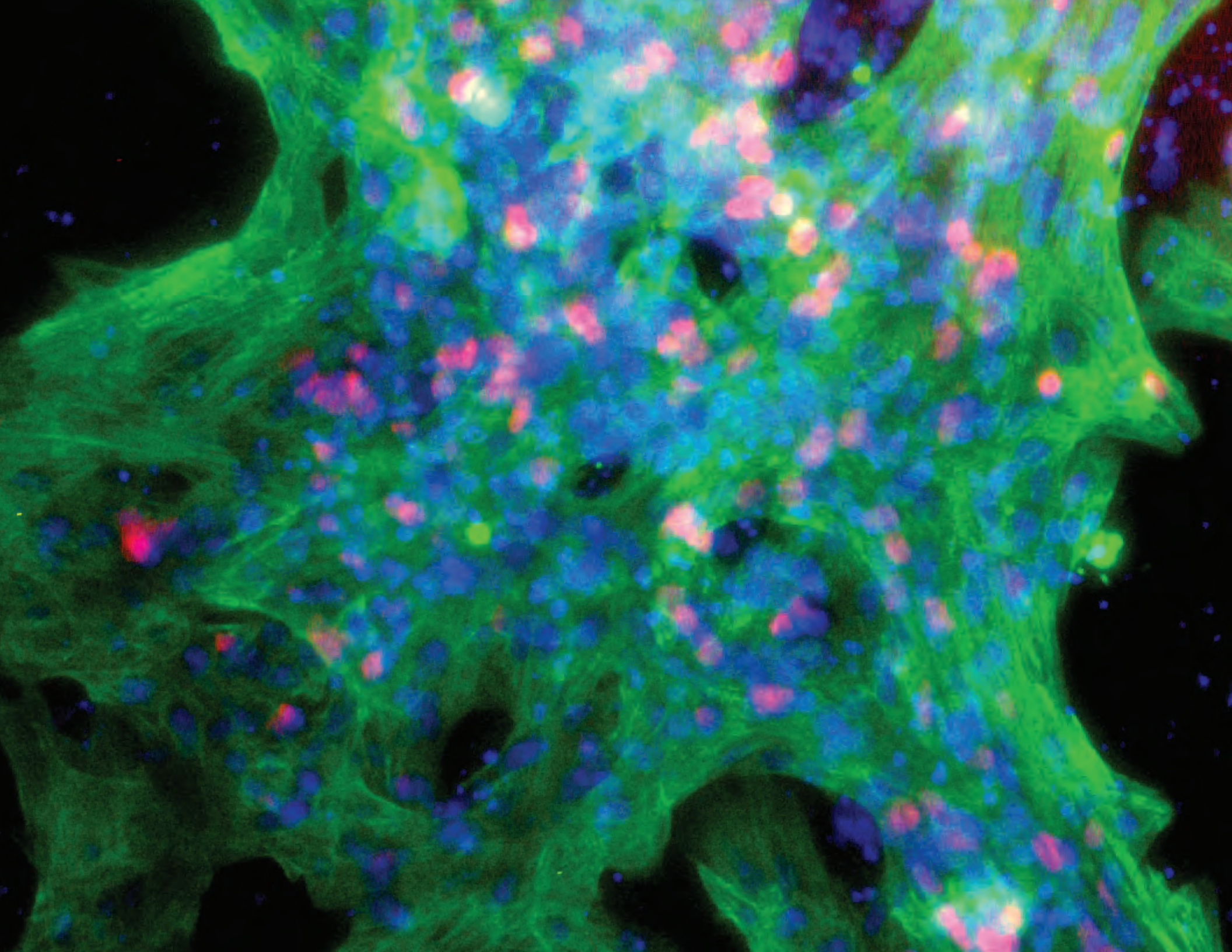


Figure 29. Representative images of transfection efficiency and maintenance of pluripotency. The Gibco Human Episomal iPSC Line, adapted to StemFlex Medium, was single cell–passaged using TrypLE Select enzyme and seeded in StemFlex Medium with 1X RevitaCell Supplement at 50,000 viable cells/cm^2 into a 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-hour 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.



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 simplifies your workflow and provides you with more control—allowing for faster, more efficient systems.

To view the complete differentiation portfolio, go to **thermofisher.com/differentiation**

Support resources

- View differentiation protocols at **thermofisher.com/stemcellprotocols**
- Request a copy of the 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 Plus Neuronal Culture System	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 NSC 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?
We have a dedicated team of stem cell scientists to help you achieve your project goals.
See page 52 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 30) 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 31). 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 32).

For more information, go to **thermofisher.com/nscdiff**

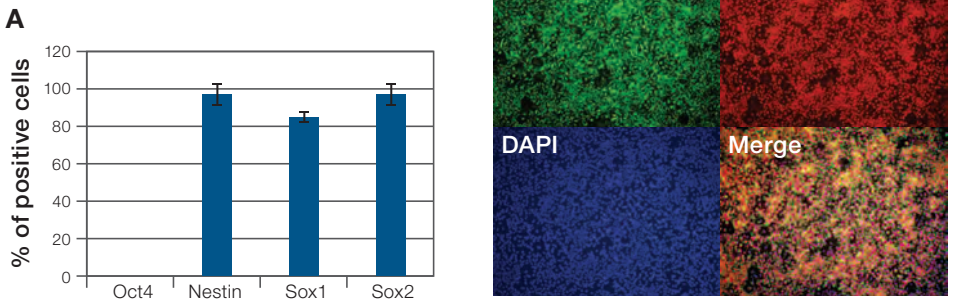


Figure 30. NSCs generated using PSC Neural Induction Medium express high levels of NSC markers nestin, Sox1, and Sox2, and low levels of residual pluripotent marker Oct4. **(A)** 80–90% neural induction efficiency. **(B)** Immunocytochemistry staining images of relevant NSC markers.

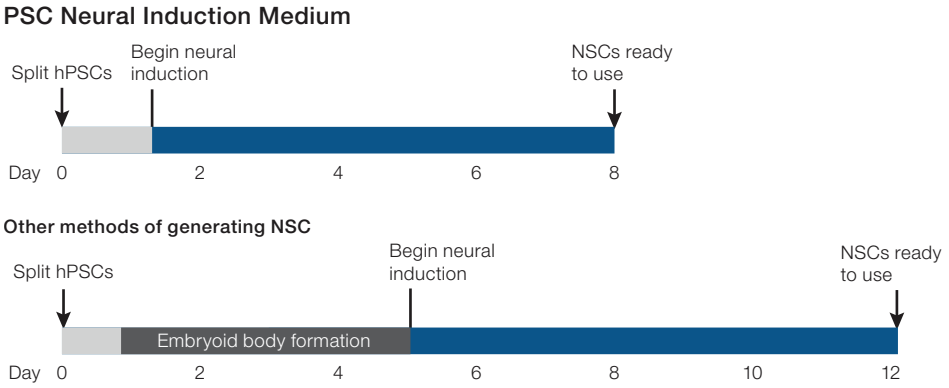


Figure 31. Unlike existing methodologies, PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation which adds time, labor, and variability.

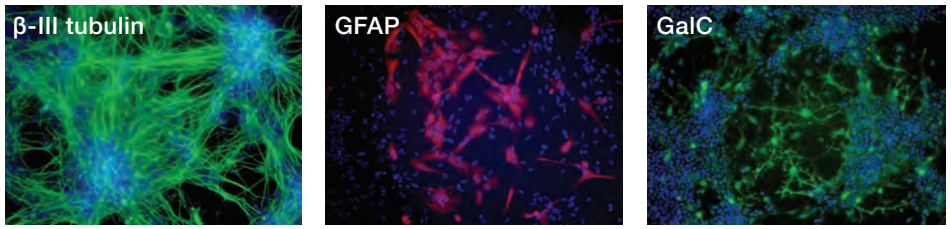


Figure 32. Neural stem cells (NSCs) generated using PSC Neural Induction Medium have high expression of NSC markers and can be further differentiated into other neural cell types.

CultureOne Supplement with B-27 Plus Neuronal Culture System

Superior neuronal cell cultures

Gibco™ CultureOne™ Supplement with B-27 Plus Neuronal Culture System significantly improves the differentiation of neural stem cells (NSCs) to neurons. As compared to conventional differentiation methods where NSCs can overgrow and become burdensome, 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 33).

For more information, go to **thermofisher.com/cultureone**

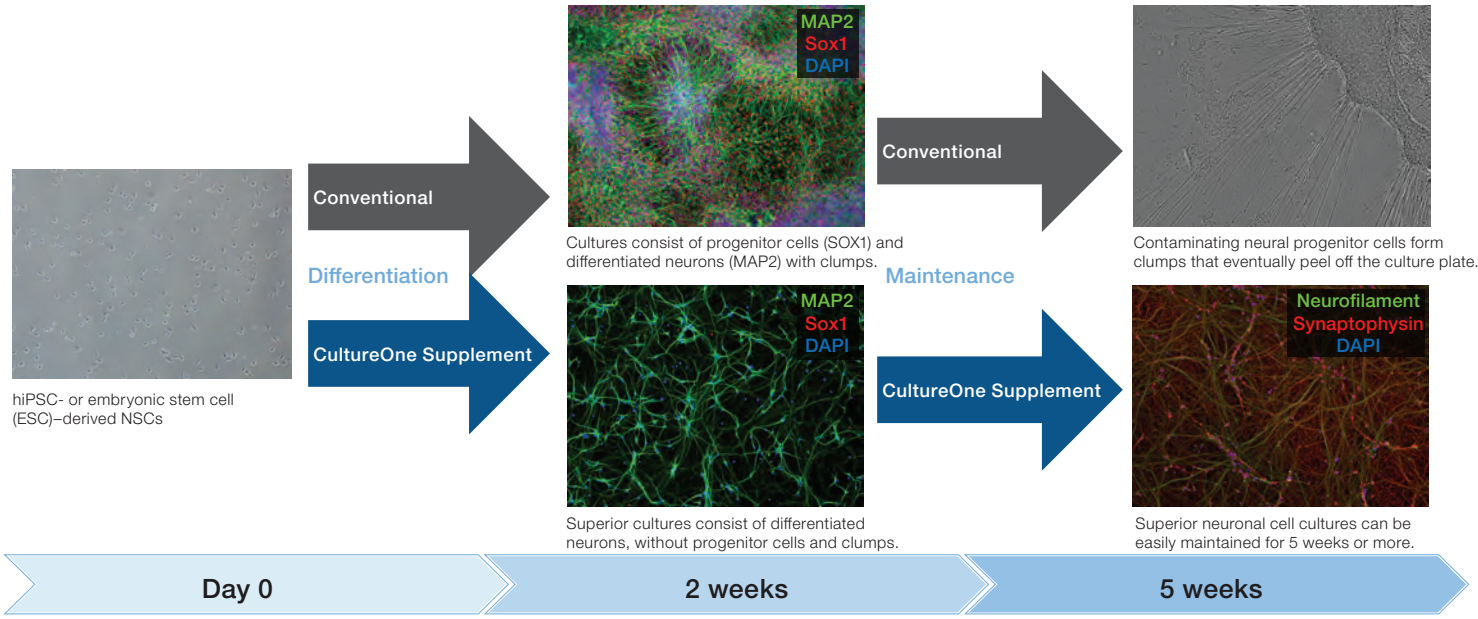


Figure 33. H9 ESC–derived NSCs were plated at a density of 5 x 10^4 cells/cm^2. Without CultureOne Supplement, cells at 2 weeks of differentiation were highly dense, formed cell clumps, and contained MAP2-positive neurons and a significant number of Sox1-positive NSCs. At 2 weeks of differentiation, 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).

PSC Dopaminergic Neuron Differentiation Kit

Differentiate iPSCs to functional midbrain dopaminergic neurons

The Gibco™ PSC Dopaminergic Neuron Differentiation Kit enables the differentiation of pluripotent stem cells (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 hPSC to midbrain floor plate cells, (2) expand and cryopreserve specified cells, and (3) revive and mature cells to midbran dopaminergic neurons (Figures 34 and 35).

For more information, go to **thermofisher.com/dopadiff**

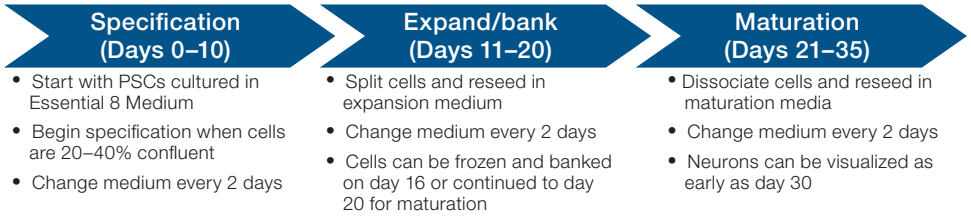


Figure 34. Pluripotent stem cells cultured in Essential 8 Medium. PSCs can be specified to the midbrain floor plate, expanded, and banked, then matured to midbrain dopaminergic neurons in 35 days. Floor plate–derived midbrain progenitors can be expanded up to 10 passages.

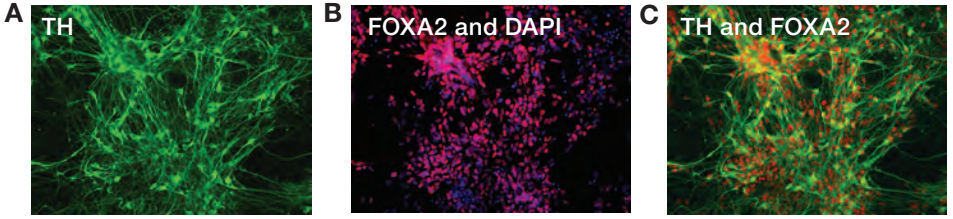


Figure 35. Representative images of mature dopaminergic neurons. The images were obtained from cells stained with reagents provided in the Invitrogen™ Human Dopaminergic Neuron Immunocytochemistry Kit (Cat. No. A29515) after 14 days of maturation of floor plate progenitor cells in Dopaminergic Neuron 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 36).

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 37), contract in culture, and can be subsequently maintained in culture for more than 15 days.

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

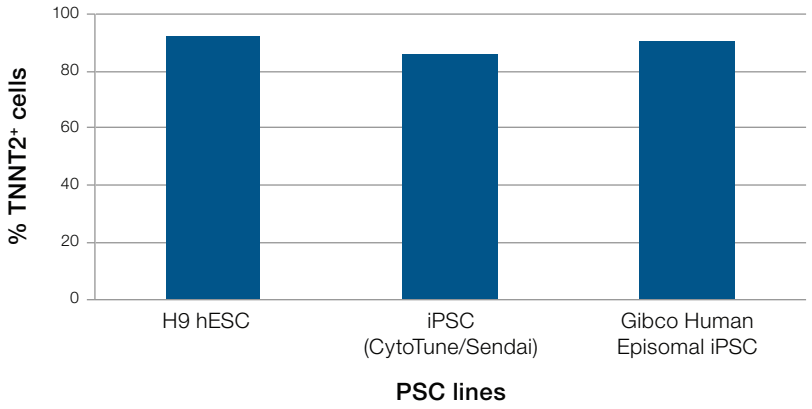


Figure 36. Efficiency across multiple PSC lines. Gibco™ TrypLE™-dissociated PSC lines were seeded at specific density onto a Geltrex-coated surface and cultured in Essential 8 Medium. After three days of expansion, PSC lines at optimal confluency were induced using the PSC Cardiomyocyte Differentiation 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 reaches optimal confluency at time of induction.

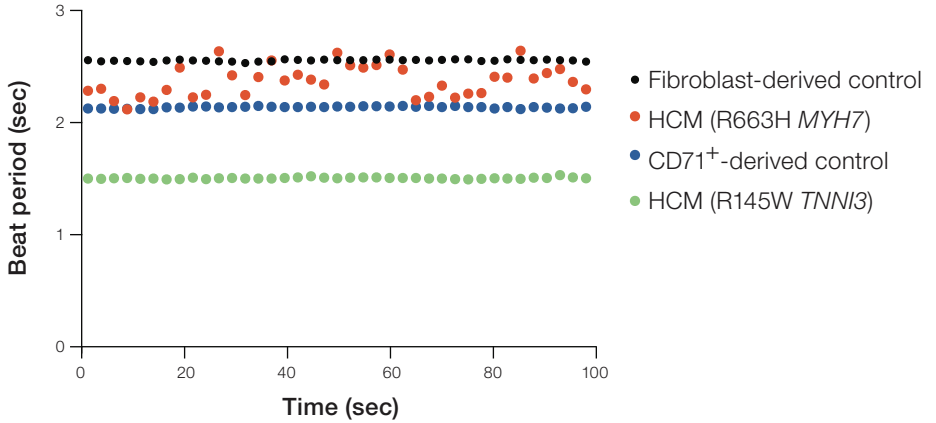


Figure 37. Electrophysiological assessment of hypertrophic cardiomyopathy patients' iPSC-derived cardiomyocytes generated using the PSC Cardiomyocyte Differentiation Kit on the Maestro™ Multielectrode Array (MEA) platform (Axion Biosystems). The arrhythmic beating of the cardiomyocytes with mutation 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 pluripotent stem cells 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 38 and 39).

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 40) and are capable of differentiating to downstream lineages.

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

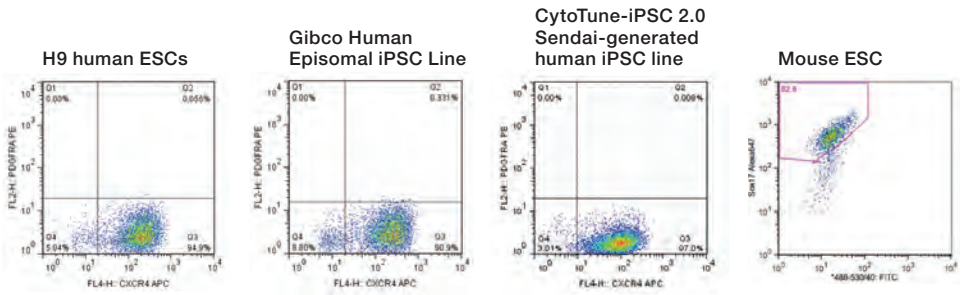


Figure 38. The PSC Definitive Endoderm Induction Kit produces DE populations with high efficiency across hESC, hiPSC, and mESC lines. hiPSCs tested include cell lines reprogrammed using episomal vectors or the CytoTune kit. Representative dot plots for hESCs and hiPSCs show CXCR4⁺/PDGFRα⁻ cell populations 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.

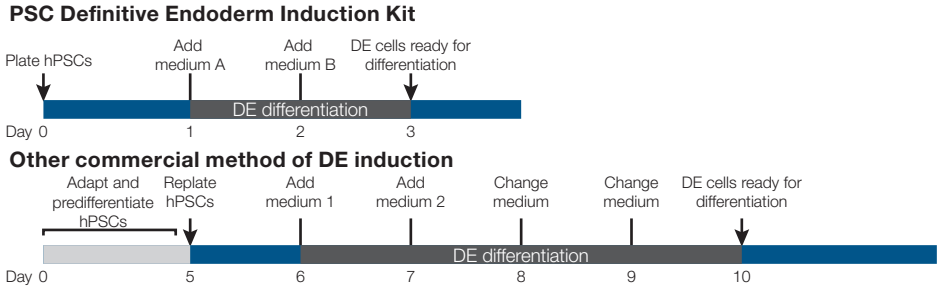


Figure 39. Compared to other differentiation protocols, the PSC Definitive Endoderm Induction Kit produces cells in up to 50% less time and requires no predifferentiation or mixing of media.

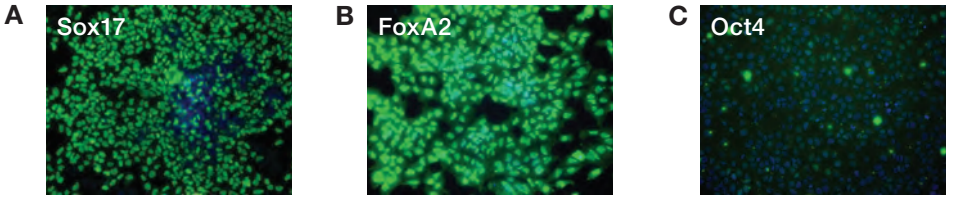
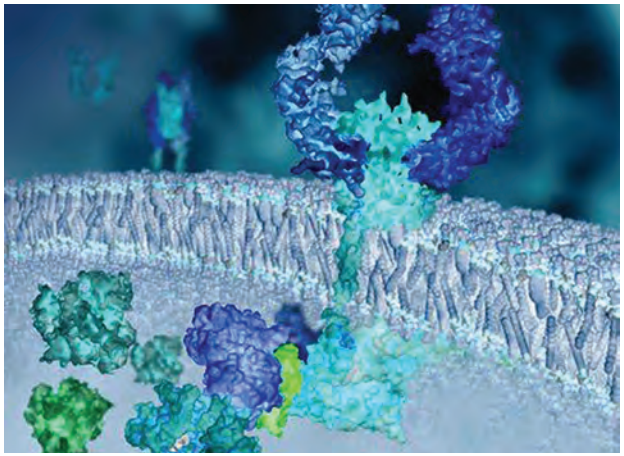


Figure 40. Immunocytochemistry of hESCs treated with the PSC Definitive Endoderm Induction Kit. At day 3, induced cells were immunostained for the endodermal transcription factors **(A)** Sox17 and **(B)** FoxA2, and the pluripotent marker **(C)** Oct4. Nuclei were counterstained with DAPI (blue) to assess total cell numbers.

Differentiation growth factors

Growth factors can stimulate stem cell differentiation and influence the stem cell developmental fate. Our high-quality Gibco™ growth factors are designed to give you high biological activity, high purity (95% pure), and <0.1 ng endotoxin per microgram. Our growth factors are verified with Gibco™ media to have proven compatibility.

In addition, our Gibco CTS growth factors are designed for use in cell and gene therapy research applications with additional safety testing and regulatory documentation to help you advance your therapy from the bench to the clinic.



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.

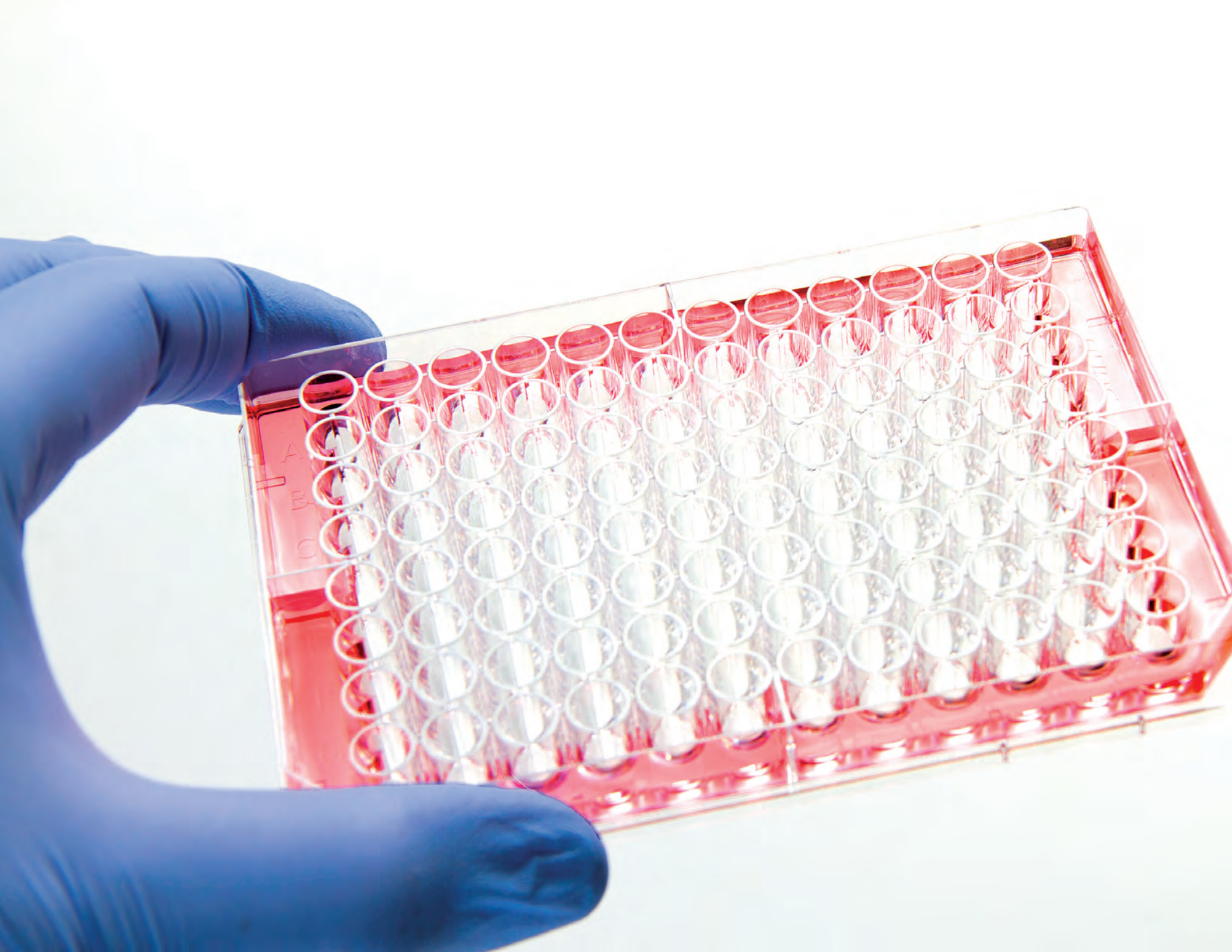
Granulocyte-macrophage colony-stimulating factor (GM-CSF)
GM-CSF is involved in many biological responses, including the growth and development of granulocyte and macrophage progenitor cells, stimulation and the initiation of differentiation of myeloblasts and monoblasts, and chemotaxis of eosinophils.

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**



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.

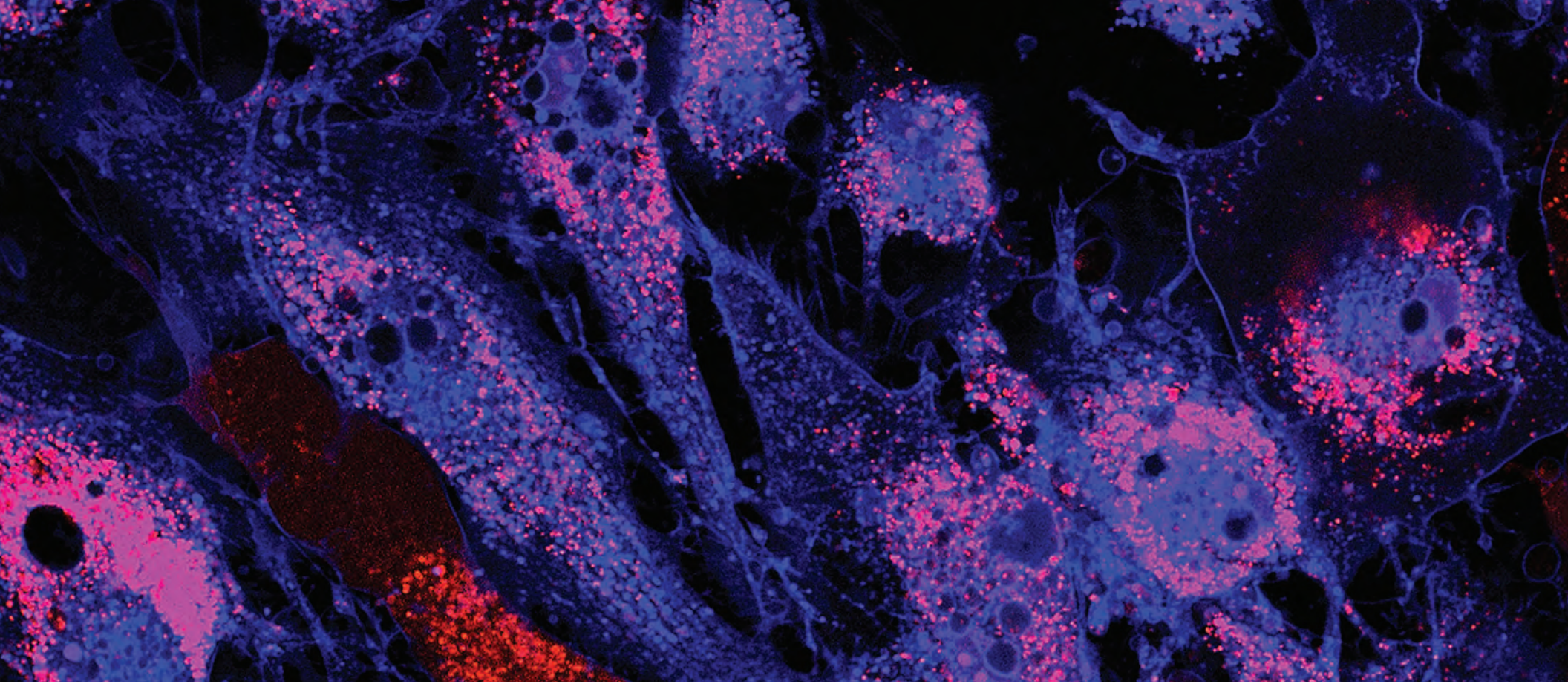
Choose the best solutions for your stem cell workflow at **thermofisher.com/cellcultureplastics**

Support resources

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

Table 13. Cell culture plastics overview.

Stem cell workflow	Cell culture format	Cell culture surface modifications						
		Standard tissue-culture treated (Nunclon Delta)	Nunclon Sphera	Collagen I	Poly-D-lysine	Untreated	Nunc UpCell	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	Lab-Tek and Lab-Tek II Chamber Slides and Chambered Coverglasses	•						•
	Nunc Optical Bottom Plates	•				•		•
	Nunc Glass Bottom Dishes	•						



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**

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
- Invitrogen™ multiplex assays
- Invitrogen™ antibodies for western detection
- Invitrogen™ ELISA kits
- Invitrogen™ Bolt™ protein separation and detection system
- 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™ AuthentiFiler™ PCR Amplification Kit
- PluriTest-compatible PrimeView Global Gene Expression Profile Assays
- KaryoStat Assays
- Applied Biosystems™ TaqMan® Gene Expression Assays
- Applied Biosystems™ TaqMan® miRNA Assays
- Applied Biosystems™ TaqMan® SNP Assays
- Applied Biosystems™ TaqMan® CNV Assays
- Ion AmpliSeq™ panels

Find your antibody match

With over 40,000 antibodies covering many stem cell targets, we can offer the best antibody for your research.
Find antibodies for all stem cell targets at **thermofisher.com/antibodies**

Selected instruments for stem cell characterization and analysis

EVOS cell imaging systems

Designed to eliminate the complexities of microscopy without compromising performance, the Invitrogen™ EVOS™ line of cell imaging systems makes cell imaging accessible to almost every lab and budget. Determine which cell imaging system is right for you at **thermofisher.com/evos**

Countess II FL Automated Cell Counter

With the option 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, monitor fluorescent protein expression, and measure cell viability in as little as 10 seconds. Designed with flexibility in mind, the Countess II FL instrument can be configured to use a full range of light cubes that provide more than 20 fluorescence color options. Find out more about the Countess II FL instrument at **thermofisher.com/countess**

Attune NxT Flow Cytometer and Autosampler

Precision with performance, the Invitrogen™ Attune™ NxT Flow Cytometer with acoustic focusing technology is a benchtop cytometer that is configurable with up to 4 lasers and 16 parameters of detection. It provides superior sample analysis speed up to 10x faster throughput than traditional cytometers with clog-resistant engineering. Easily switch between tubes and plates in seconds and leverage the complete walk-away automation of your 96- or 384-well plates with the robotic automation–capable Attune™ Autosampler. The Attune NxT instrument is designed to enable researchers to see what wasn't visible before. See more about the Attune NxT Flow Cytometer at **thermofisher.com/attune**

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. Go to **thermofisher.com/pgm** to see more about the Ion PGM System.

Ion GeneStudio S5 series

The Ion GeneStudio™ S5 series 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**

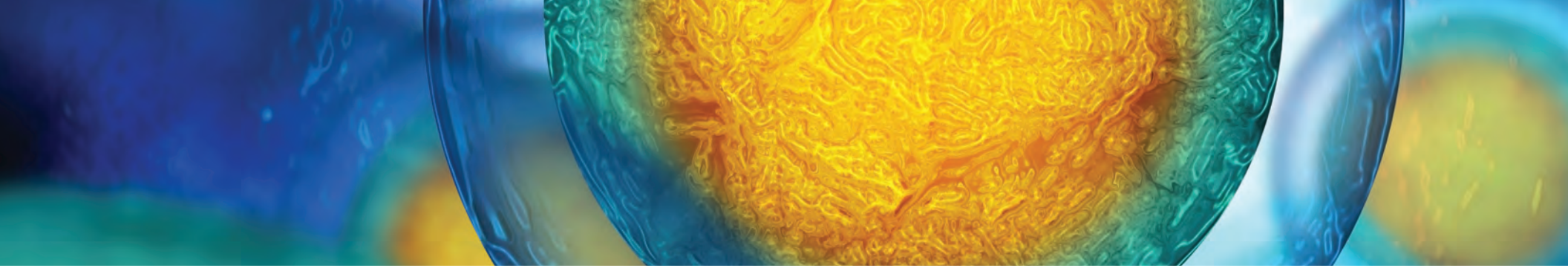
Gene Chip Scanner 3000 7G System

The Applied Biosystems™ GeneChip™ Instrument System is a fully integrated platform for conducting your research using GeneChip-brand probe arrays. The Applied 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**

GeneTitan MC Instrument

Transform your lab and experience the superior power of streamlining 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, we have 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 help you achieve a smooth transition. 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, to ease the burden on your quality systems by helping to support your regulatory submission and reduce risk throughout.

To find the best solutions and support for your pluripotent stem cell therapy needs, go to **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/ctsselectionguide**
- Access the CTS mini-documentary series videos at **thermofisher.com/cts-videoseries**

cGMP-compliant manufacturing

- Manufactured in conformity with cGMP principles
- Internal manufacturing sites are US Food and Drug Administration (FDA)–registered with an ISO 13485–certified quality management system

Testing and documentation

- Traceability documentation, including Drug Master Files and Certificates of Origin
- Extensive safety testing—including sterility, endotoxin, adventitious agent and mycoplasmas on applicable products

Proven use

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

Pluripotent stem cell therapy workflow solutions

Isolation

Isolation
(T cells) CTS Dynabeads CD3/CD28

Media
(T cells) OpTmizer medium

(PBMC/CD34$^+$ cells) StemPro-34 SFM

(Fibroblasts) CTS KnockOut SR XenoFree

Dissociation/passaging reagents
CTS TrypLE Select Enzyme

Reprogramming

Reprogramming kits
CytoTune-iPS 2.0 Sendai Reprogramming Kit

CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit

Transfection reagents
(Fibroblasts) Lipofectamine Stem 3000 Transfection Reagent

Electroporation device
(T cells) Neon Transfection System

Banking and recovery

Cryopreservation
CTS Synth-a-Freeze Medium

PSC Cryopreservation Kit

Recovery
RevitaCell Supplement

CTS Essential 8 Medium/ CTS Vitronectin

rhLaminin-521

Expansion and gene editing

Expansion media systems
CTS KnockOut SR XenoFree

CTS KnockOut DMEM/F-12

CTS CELLstart Substrate

CTS KnockOut DMEM

CTS Essential 8 Medium/ CTS Vitronectin

EDTA/Versene Solution

Custom media and services

Gene editing
CRISPR-Cas9 products and services

Designer TALEN products and services

Lipofectamine Stem Transfection Reagent

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 B-27 Supplement, XenoFree

CTS B-27 Supplement, XenoFree, Minus Vitamin-A

CTS B-27 Supplement, XenoFree, Minus Insulin

CTS N-2 Supplement

CTS KnockOut DMEM/F-12

CTS Neurobasal Medium

CTS Neurobasal-A Medium

CultureOne Supplement

B-27 Plus Neuronal Culture System

Growth factors
CTS TGF-β 1

CTS Stem Cell Factor (SCF)

CTS FLT 3 Ligand

CTS FGF-Basic, Full Length

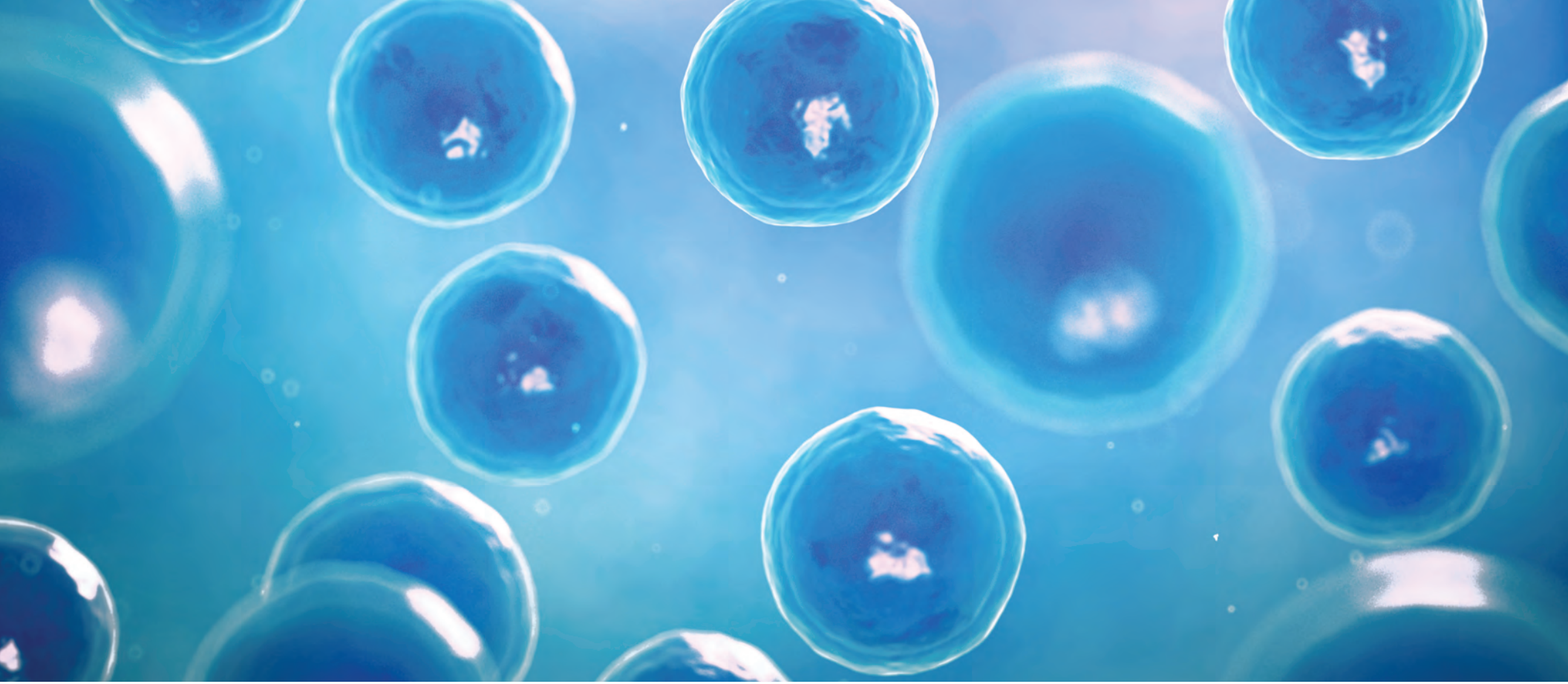
Wash, fill, finish, and cryo

Wash
CTS DPBS

Cryopreservation medium
CTS Synth-a-Freeze Medium

Cryogenic storage and logistics
Banking services

Cold-chain logistics solutions



Services and support

Built on the stem cell innovations that we have introduced throughout the past decade, our Gibco™ CellModel™ Services 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 choices at every stage of the stem cell workflow. Choose the services that best fit your research needs.

Sample

Reprogramming

Reprogram your human fibroblasts or blood cells to PSCs

Reprogramming kits
- CytoTune-iPS 2.0 Sendai Reprogramming Kit
- Epi5 Episomal iPSC Reprogramming Kit

Genome editing

Introduce disease-relevant mutations or indels to your PSCs

Tools and reagents
- TrueCut Cas9 Protein v2
- TrueGuide Synthetic gRNA
- PerfectMatch TAL Effectors
- Human episomal Cas9 iPSC cell line
- Lipofectamine 3000 Stem Transfection Reagent

Culture/ cryopreservation

Culture and cryopreserve your PSC cell banks

Media
- Essential 8 Flex Medium
- Essential 8 Medium
- StemFlex Medium

Supplements
- RevitaCell Supplement

Differentiation

Differentiate your PSCs to your desired terminal cell type

Differentiation kits
- PSC Cardiomyocyte Differentiation Kit
- PSC Definitive Endoderm Induction Kit
- PSC Dopaminergic Neuron Differentiation Kit
- PSC Neural Induction Medium

Assay development

Develop and optimize custom assays to support drug discovery and development

Assays
- BacMam assay
- CellSensor assay
- Fluo-4 assay
- GeneBLAzer assay
- PrestoBlue assay
- TaqMan assay

Screening

Screen compounds using our high-throughput screening facility

Services
- SelectScreen Services

Characterization

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

Tools
- Protein analysis
- PluriTest-compatible PrimeView Global Gene Expression Profile Assays
- KaryoStat Assays
- Sequencing/RT-qPCR
- TaqMan hPSC Scorecard Panel
- Immunocytochemistry kits

Instrumentation
- CellInsight CX7 HCA instrument
- QuantStudio systems
- EVOS cell imaging instruments
- Ion PGM sequencer
- GeneChip Scanner 3000 7G System

To inquire about other services or instrumentation, go to **thermofisher.com/askdiscovery**

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
- 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:

- Dedicated team of stem cell scientists to deliver results on your project
- Detailed protocols provided to you after project completion to demonstrate how we reached each milestone and document the tools we utilized
- 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 and frequent project communication provided by a team with extensive experience delivering custom services

David Piper
Senior Manager,
Custom Biology R&D,
Thermo Fisher Scientific

"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 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

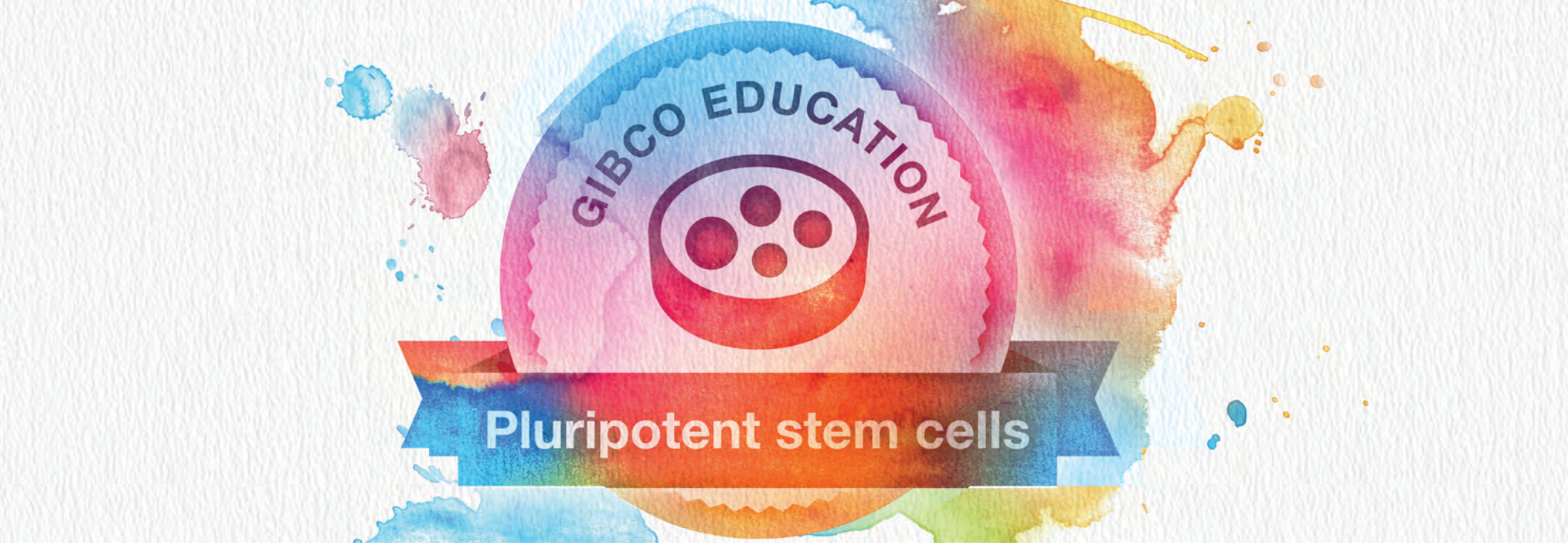
After the "correction" of the disease-relevant mutations, the team expanded and cryopreserved the edited PSCs to generate a suitably sized bank of cells to support further R&D activities.

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



Pluripotent stem cell education

Whether you are looking to expand, test, or apply your stem cell knowledge, we have the educational tools for you. We offer everything you need, in formats that fit all learning preferences, to enable your success and empower your growth.

Take control of your education at **thermofisher.com/psceducation**

Expand your knowledge with:

- Key assets such as our Pluripotent Stem Cell Resource Handbook and Pluripotent Stem Cell Protocol Handbook
- Key industry events such as ISSCR and Gibco™ 24 Hours of Stem Cells

Test your knowledge with:

- Gibco™ PSC Culture Virtual Lab
- Hands-on Gibco™ Pluripotent Stem Cell Workshop

Apply your knowledge with:

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

Pluripotent Stem Cell Workshops

We have proudly established Gibco™ Stem Cell Research Centers in Carlsbad, California, USA; Frederick, Maryland, USA; and Glasgow, Scotland, UK. These centers provide customers with hands-on stem cell training in techniques for culturing and characterizing human embryonic stem cells and induced pluripotent stem cells, as well as reprogramming techniques for the creation of iPSCs. Whether you're new to pluripotent stem cell research or need a refresher course, our R&D scientists can provide detailed stem cell training so you can feel confident using stem cells in your research.

Get more information on the training courses, including registration and this year's course dates, at **thermofisher.com/pscworkshop**

Training course agenda topics include:

- Basic maintenance and care of hESCs and iPSCs
- Freezing, thawing, plating, and passaging techniques
- Culturing PSCs under feeder-dependent and feeder-free conditions
- Reprogramming and identification of iPSCs
- Differentiation and characterization methods for PSCs

Specialized training support:

Each training workshop is structured as a three-day course with both lecture and hands-on laboratory work. Our specialized, experienced trainers will guide you through a variety of stem cell techniques and work with you one-on-one to help ensure your success.

Hands-on CRISPR training course

The powerful gene-editing technology known as CRISPR has the potential to transform science at an astonishingly rapid rate. At Thermo Fisher Scientific, we are committed to helping you stay ahead and advance your science through education. Our experienced team has designed a comprehensive four-day CRISPR workshop comprising both lectures and hands-on laboratory work at our state-of-the-art training facility.

Find out more or register today at **thermofisher.com/crisprworkshop**

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 CD34+ Cell Kit	A14059
StemPro Human Adipose-Derived Stem Cell Kit	R7788110
StemPro Human Adipose-Derived Stem Cells	R7788115
StemPro MSC SFM XenoFree	A10675-01
StemPro Neural Stem Cells	A15654
StemPro NSC SFM	A1050901
TrueCut Cas9 Protein v2	A36498
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
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 Instrument	MPK5000S
Genome editing	
Cas9 iPSC	Contact GEMServices@thermofisher.com
Cas9 stable cell line	
GeneArt CRISPR Nuclease mRNA	A29378
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit	A21175
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit (with competent cells)	A21177
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit	A21174
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit (with competent cells)	A21178

Product	Cat. No.
GeneArt Genomic Cleavage Detection Kit	A24372
GeneArt Genomic Cleavage Selection Kit	A27663
GeneArt Precision gRNA Synthesis Kit	A29377
Introduction to CRISPR-Cas9 Genome Editing Hands-On Workshop	A33133
LentiArray Cas9 Lentivirus	A32064
LentiArray Cas9 Lentivirus	A32069
LentiArray Lentiviral sgRNA	A32042
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	A32062
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	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
TrueCut Cas9 Protein v2	A36498
TrueGuide sgRNA	A32044
Culture	
Collagenase IV	17104-019
CTS Essential 8 Medium	A2656101
CTS TrypLE Select Enzyme**	A12859-01
Essential 8 Adaptation Kit	A25935
Essential 6 Medium	A1516401
Essential 8 Flex Medium Kit	A2858501
Essential 8 Medium	A1517001
KnockOut DMEM	10829018
KnockOut Serum Replacement†	10828-028
KnockOut Serum Replacement – Multi-Species	A31815-02
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
TrypLE Express Enzyme (1X), no phenol red	12604013
TrypLE Select Enzyme (1X), no phenol red	12563011

Product	Cat. No.
Matrices and feeder cells	
CTS CELLstart Substrate**	A10142-01
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
Gibco B6-Puro Mouse Embryonic Fibroblasts, Irradiated	A34965
Gibco CF1 Mouse Embryonic Fibroblasts, Irradiated	A34181
Gibco CF1 Mouse Embryonic Fibroblasts, MitC-Treated	A34959
Gibco C57BL/6 Mouse Embryonic Fibroblasts, MitC-Treated	A34962
Gibco CF6-Neo Mouse Embryonic Fibroblasts, Irradiated	A34963
Gibco CF6-Neo Mouse Embryonic Fibroblasts, MitC-Treated	A34964
Gibco DR4 Mouse Embryonic Fibroblasts, Irradiated	A34966
Gibco Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903
rhLaminin-521	A29248
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Cryopreservation	
CTS Synth-a-Freeze Cryopreservation Medium**	A13713-01
PSC Cryopreservation Kit	A2644601
Synth-a-Freeze Cryopreservation Medium	A12542-01
Differentiation	
Activin A Recombinant Human Protein	PHC9564
B-27 Plus Neuronal Culture System	A3653401
B-27 Supplement (50X), serum free	17504044
bFGF Recombinant Human Protein	13256029
CultureOne Supplement	A3320201
EGF Recombinant Human Protein	PHG0311
GM-CSF Recombinant Human Protein	PHC2015
Neurobasal Medium	21103049
PSC Cardiomyocyte Differentiation Kit	A2921201

Product	Cat. No.
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	
3–Germ Layer Immunocytochemistry Kit	A25538
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

Unless otherwise indicated, all products are For Research Use Only. Not for use in diagnostic procedures.
* For *In Vitro* Diagnostic Use.
** For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. Caution: Not intended for direct administration into humans or animals.
† 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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Find out more at **thermofisher.com/stemcells**

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