

# Pluripotent stem cell guidebook

Key products and services for PSC research

# The pluripotent stem cell workflow



EVOS cell imaging systems  
Attune NxT Flow Cytometer  
TaqMan hPSC Scorecard Panel  
CellInsight CX7 High Content Screening (HCS) platform

[thermofisher.com/detectpsc](https://www.thermofisher.com/detectpsc)

Countess II and Countess II FL Automated Cell Counters  
Immunocytochemistry and live staining kits  
GeneArt Genomic Cleavage Detection Kit  
GeneArt Genomic Cleavage Selection Kit

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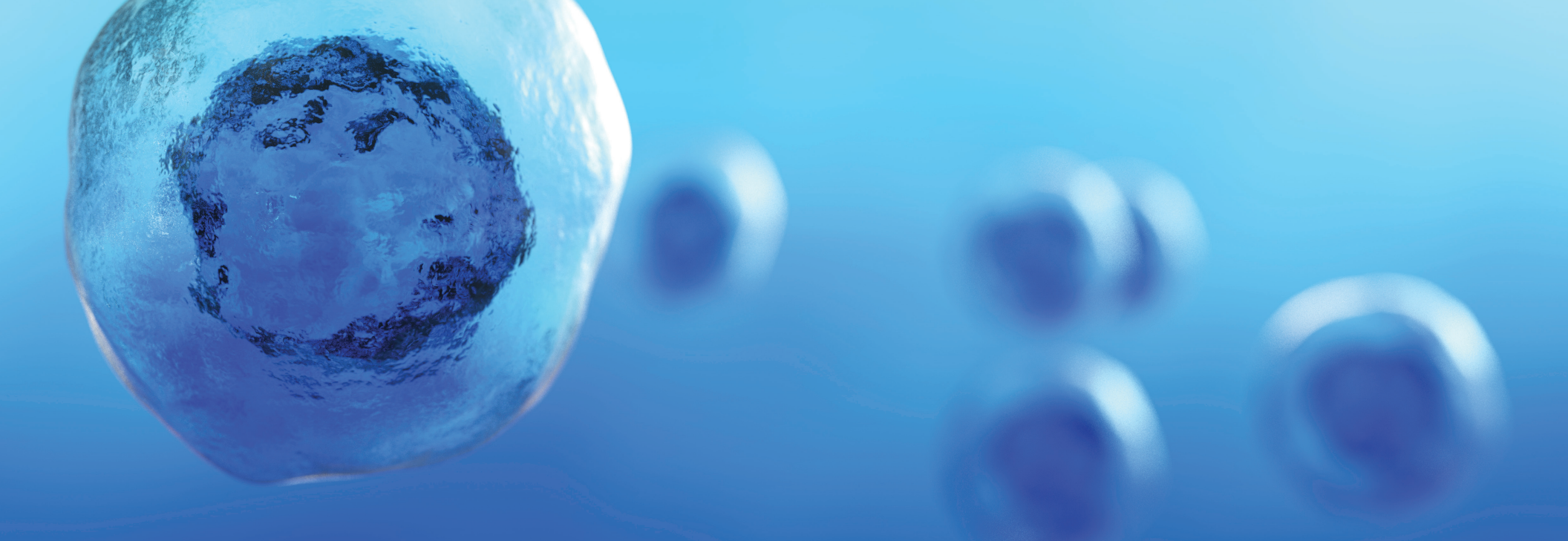
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## Supporting research from somatic to differentiated cells

Human pluripotent stem cell research holds tremendous potential in the areas of developmental biology, disease modeling, and cell therapy. 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 cell 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 learn more about products and services at [thermofisher.com/stemcells](https://thermofisher.com/stemcells)

## SUPPORT RESOURCES:

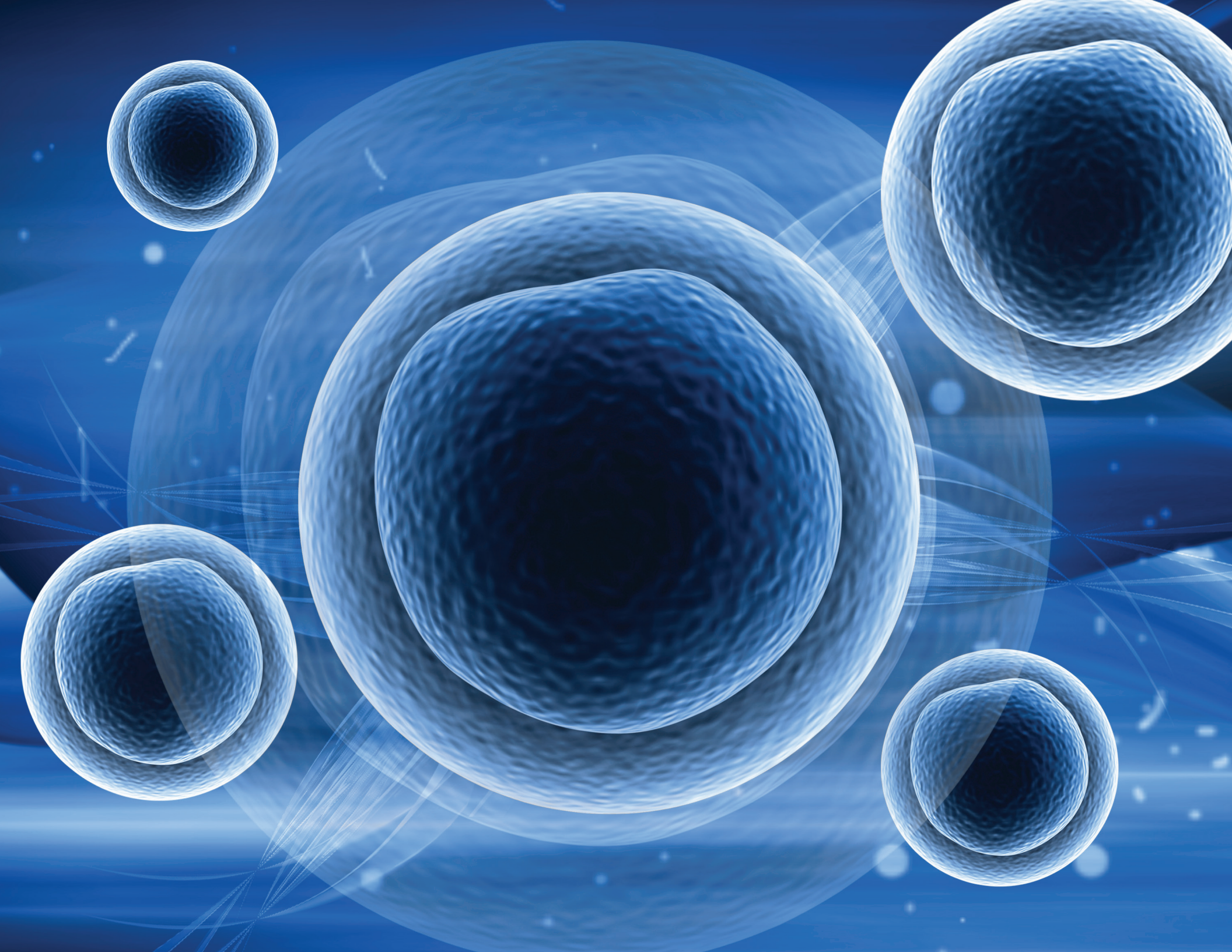
- To request the MSC\* Sourcebook, a product reference guide supporting your MSC/ADSC workflow, go to [thermofisher.com/mscbook](https://thermofisher.com/mscbook)
- View stem cell protocols for expanding somatic cells at [thermofisher.com/stemcellprotocols](https://thermofisher.com/stemcellprotocols)

**Table 1. Somatic and progenitor cell media overview.**

Cell type	ADSC*	MSC*	CD34 <sup>+</sup> and PBMC*	PBMC	T cell	NSC*	Human fibroblast
<b>Human adult stem and primary cells</b>	StemPro Human Adipose-Derived Stem Cells	StemPro BM Mesenchymal Stem Cells	StemPro CD34 <sup>+</sup> Cell Kit	N/A	N/A	StemPro Neural Stem Cells	Human Dermal Fibroblasts, Adult
<b>Recommended culture media</b>	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
<b>GMP compliance</b>	Media	Media and cells	Media	Media	Media	Media and cells	Media
<b>Application</b>	Reduces doubling times and variability of ADSCs	Xeno-free medium for human ADSC and MSC expansion	Supports CD34 <sup>+</sup> 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
<b>Antibodies</b>	Find antibodies for all stem cell targets at <a href="https://thermofisher.com/antibodies">thermofisher.com/antibodies</a>						

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

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





Reprogramming somatic cells to 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](https://thermofisher.com/reprogramming) to find the best solution for your reprogramming experiment.

## SUPPORT RESOURCES:

- View cell reprogramming protocols at [thermofisher.com/stemcellprotocols](https://thermofisher.com/stemcellprotocols)
- Access technical resources for CytoTune-iPS Kits at [thermofisher.com/cytotuneresources](https://thermofisher.com/cytotuneresources)

**Table 2. Non-integrating 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
<b>Applications</b>	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
<b>Reprogramming efficiency</b>	0.002–0.08%	0.04–0.3%	0.02–1.2%	0.01–0.6%
<b>Genes utilized</b>	Thomson/Yamanaka factors	Yamanaka factors + Lin28	Yamanaka factors	Yamanaka factors (L-myc replaces c-myc)
<b>Blood reprogramming</b>	Yes (with Neon system only)	Yes (with Neon system only)	Yes	Yes
<b>Delivery method</b>	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 45 for all of our stem cell services.

# CytoTune-iPS 2.0 Sendai Reprogramming Kit

## High success rate among non-integrating 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, visit [thermofisher.com/cytotune](https://thermofisher.com/cytotune)

**Table 3. Human somatic cell types that have been successfully reprogrammed with CytoTune kits.**

Human		Mouse
Adult and neonatal dermal fibroblasts	Peripheral blood mononuclear cells (PBMCs)	Mouse embryonic fibroblasts
Amniotic fluid MSCs	Skeletal myoblasts	
Cardiac fibroblasts	T cells	
CD34 <sup>+</sup> blood cells	Umbilical vein epithelial cells	
Mammary epithelial cells	Urine epithelial cells	
Nasal epithelial cells		

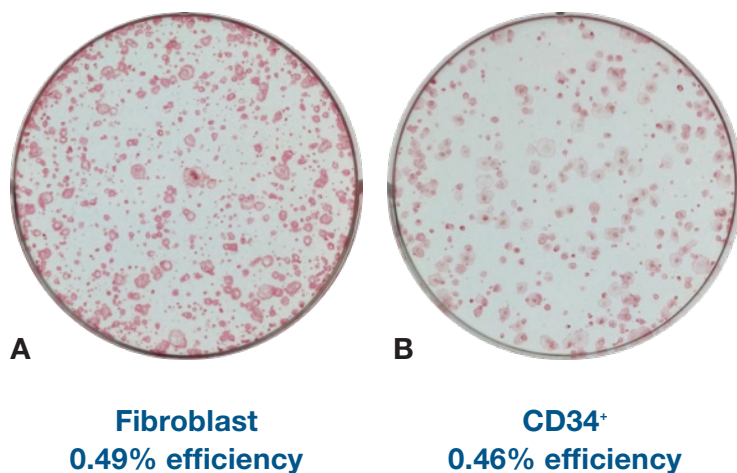
For publications citing Sendai virus for iPSC generation, visit [thermofisher.com/sendapubs](https://thermofisher.com/sendapubs)



## Seamless transition to the clinic Invitrogen™ CTS CytoTune™-iPSC 2.1 Sendai Reprogramming Kit

- First off-the-shelf reprogramming system manufactured under GMP principles
- Xeno-free workflow for generation of iPSC lines from both fibroblasts and blood for clinical research
- CTS CytoTune kit offers the high-efficiency Sendai delivery of reprogramming factors, and extensive testing and documentation to support your regulatory submission

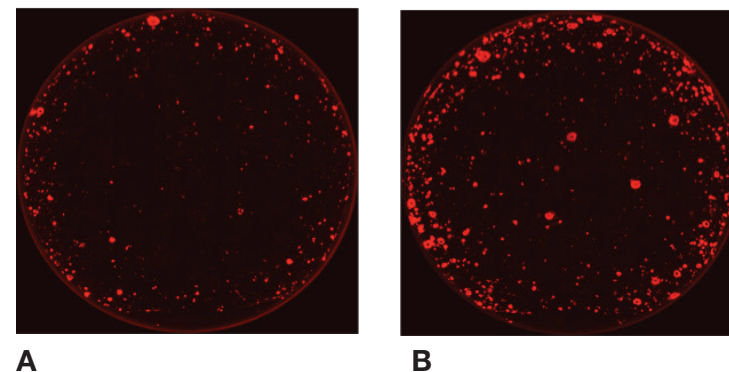




**Figure 1. Reprogramming efficiency.** Alkaline phosphatase staining of iPSCs generated from (A) human dermal neonatal fibroblasts (BJ strain) and (B) human umbilical cord blood–derived CD34<sup>+</sup> cells, using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at an MOI of 5:5:3, shown at 21 days posttransduction.

### Need even better reprogramming efficiency?

Supplement PSC culture media on day 7 of reprogramming with Gibco™ RevitaCell™ Supplement.



**Figure 2. Improvement of feeder-free reprogramming efficiency using RevitaCell Supplement on day 7 transfer.** Feeder-free reprogramming of human dermal neonatal fibroblasts (HDFn) (Cat. No. C-004-5C) was completed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at an MOI of 5:5:3. On day 7 posttransduction, reprogrammed fibroblasts were transferred to rhVTN-N matrix in growth medium in the (A) absence and (B) presence of RevitaCell Supplement for 24 hours posttransfer followed by daily feeding with Gibco™ Essential 8™ Medium alone.

### Alkaline Phosphatase Live Stain

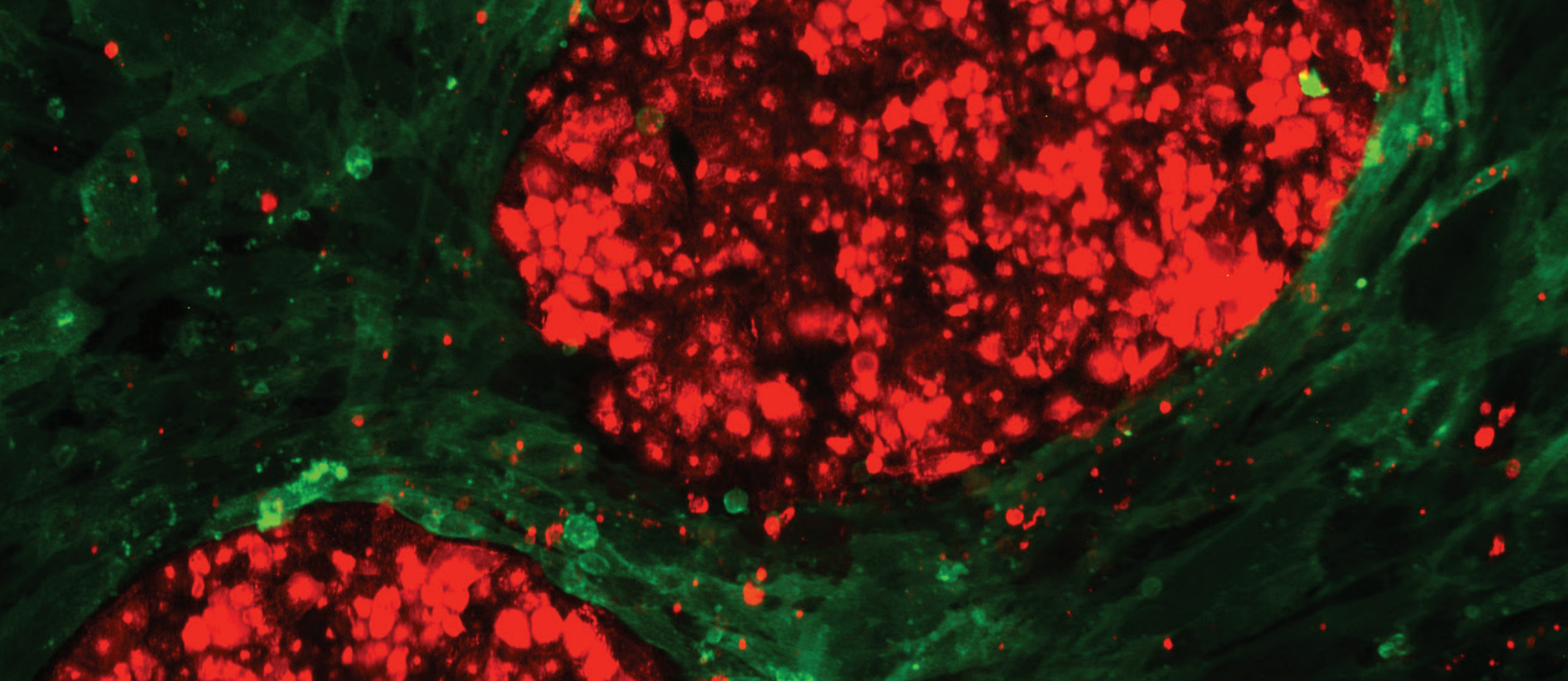
Invitrogen™ Alkaline Phosphatase Live Stain is used for stem cell imaging that allows you to differentially stain PSCs. The dye is a cell-permeable fluorescent substrate for alkaline phosphatase (AP) that is nontoxic to cells, diffusing away over the course of 2 hours.

Learn more at [thermofisher.com/aplivestain](https://www.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.

Learn more at [thermofisher.com/pscimmunokits](https://www.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.

Visit [thermofisher.com/psculture](https://thermofisher.com/psculture) to find the right PSC media for your research.

### SUPPORT RESOURCES:

- View cell culture protocols at [thermofisher.com/stemcellprotocols](https://thermofisher.com/stemcellprotocols)
- Access Essential 8 Medium how-to videos at [thermofisher.com/essential8howto](https://thermofisher.com/essential8howto)



Table 4. Media systems for PSC culture.

		Feeder-dependent culture		Feeder-free culture	
<b>Medium</b>		KnockOut Serum Replacement–Multi-Species + DMEM/F-12 (for human) or KnockOut DMEM (for mouse)	CTS KnockOut SR XenoFree Medium + CTS KnockOut DMEM	StemFlex Medium	Essential 8 Media
<b>Ideal for</b>		Feeder-based human and mouse PSC culture, reprogramming, gene editing, and differentiation	Translational or clinical research applications	Robust maintenance of PSC culture, difficult cell lines, and superior performance in single-cell passaging and gene editing	Translational or clinical research applications, researchers needing to control what is in their media system, superior reprogramming
<b>Recommended cell types</b>		Mammalian PSCs	Human PSCs	Human PSCs	Human PSCs
<b>Xeno-free formulation</b>		No	Yes	No	Yes
<b>Weekend-free feeding schedule</b>		No	No	Yes	Yes, Essential 8 Flex Medium
<b>Recommended matrix</b>		Mouse Embryonic Fibroblasts and Attachment Factor (for human and mouse)	CTS CELLstart Substrate	Geltrex Matrix	Vitronectin (VTN-N) Recombinant Human Protein
<b>Other compatible matrices</b>		N/A	N/A	Vitronectin (VTN-N) Recombinant Human Protein rhLaminin-521	rhLaminin-521 Geltrex Matrix
<b>Recommended level of dissociation and passaging reagent</b>	<b>Clump</b>	Collagenase IV (for human)	Versene Solution	Versene Solution	Versene Solution
	<b>Small cluster</b>			StemPro Accutase medium	
	<b>Single cell</b>	TrypLE Express Enzyme (for mouse)	CTS TrypLE Select Enzyme	TrypLE Express Enzyme, no RevitaCell Supplement required during recovery if using rhLaminin-521	TrypLE Express Enzyme with RevitaCell Supplement added to medium during recovery
<b>Additional reagents</b>		GlutaMAX-1, NEAA, 2-mercaptoethanol, and bFGF (for human) or LIF (for mouse)	bFGF CTS and 2-mercaptoethanol	RevitaCell Supplement optional for added boost and support	RevitaCell Supplement suggested for stressful events



### 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 45 for all of our stem cell services.

# Essential 8 Media

## 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 eight essential components needed to grow and expand PSCs. Many feeder-free stem cell media contain 20 or more components in their formulations (Table 6). 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 Media?

- 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
- No BSA or HSA

Learn more about the variations of Essential 8 Medium at [thermofisher.com/essential8media](https://thermofisher.com/essential8media)

**Table 5. 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	N/A
Superior recovery during transition to a defined, feeder-free culture system	Essential 8 Adaptation Kit	Kit includes rhLaminin-521
Flexible feeding schedule (including weekend-free) to eliminate daily feeding	Essential 8 Flex Medium	N/A
Supports optimum reprogramming efficiency of somatic cells due to elimination of BSA	Essential 8 Medium or Essential 8 Flex Medium	CytoTune-iPS 2.0 Sendai Reprogramming Kit
Support cells through stressful transitions in a defined media system	Essential 8 Medium or Essential 8 Flex Medium	RevitaCell Supplement
Enables more efficient embryoid body (EB) differentiation	Essential 6 Medium	N/A

**Table 6. Comparison of published PSC medium formulations.**

The formulations of STEMCELL Technologies mTeSR™1 medium and Essential 8 Medium shows significant differences in the number of components required to support PSC growth and expansion. Also of notable difference is the removal of BSA from Essential 8 media.

Components	mTeSR	Essential 8
DMEM/F12	X	X
L-ascorbic acid	X	X
Selenium	X	X
Transferrin	X	X
NaHCO <sub>3</sub>	X	X
Insulin	X	X
FGF2	X	X
TGFB1	X	X
Albumin (BSA)	X	
Glutathione	X	
L-glutamine	X	
Defined lipids	X	
Thiamine	X	
Trace elements B	X	
Trace elements C	X	
β-mercaptoethanol	X	
Pipecolic acid	X	
LiCl	X	
GABA	X	
H <sub>2</sub> O	X	





# StemFlex Medium

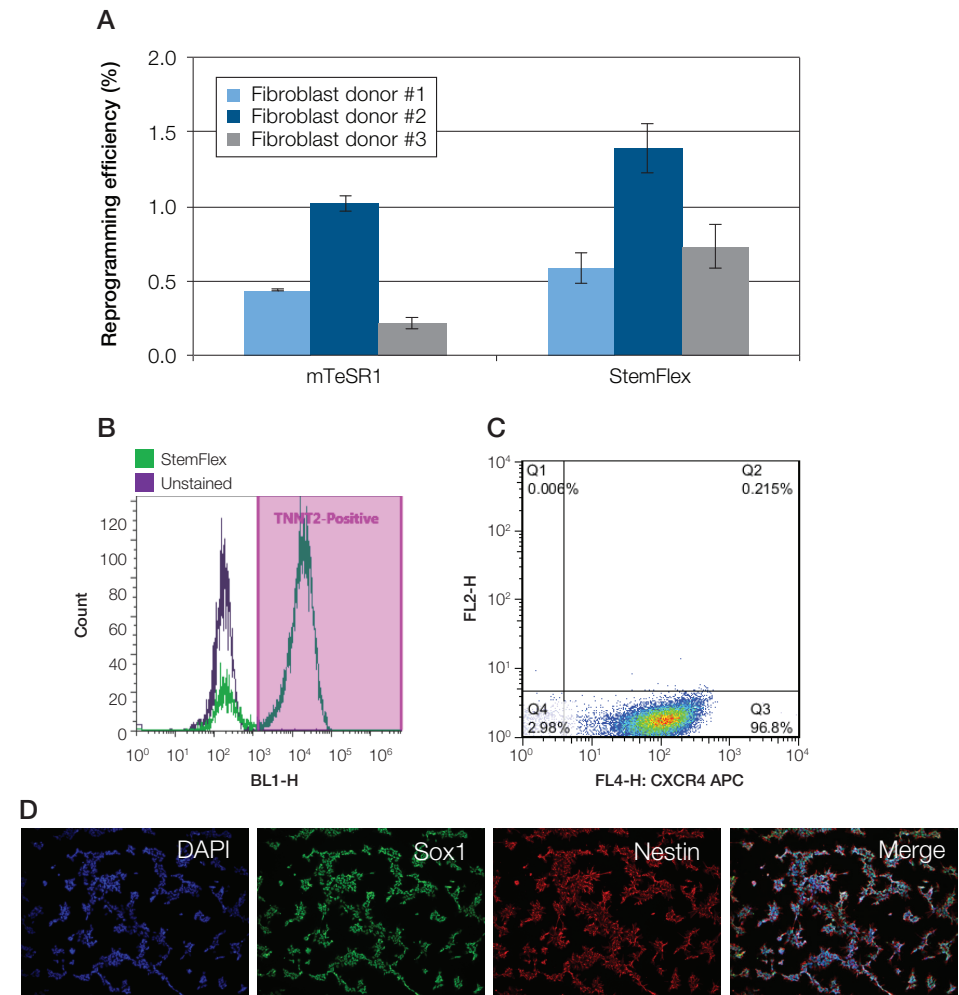
## Enhanced flexibility and superior performance in today's stem cell applications

Gibco™ 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 enables the long-term feeder-free culture of PSCs without karyotypic abnormalities and maintains cells' ability to differentiate into all three germ layers up to 50 passages (Figure 3).

### Why StemFlex Medium?

- Superior performance in gene editing, single-cell passaging, and other stressful applications (see page 29, Figure 14)
- Out-of-the-box solution with no optimization or additional reagents required
- 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](https://thermofisher.com/stemflex)



**Figure 3. StemFlex medium provides a robust formulation that can be applied across the entire PSC workflow—from somatic cell reprogramming (A) through downstream differentiation (D).** When compared to traditional feeder-free media, like mTeSR1, StemFlex 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 expression of the CXCR4<sup>+</sup>, PDGFRα<sup>+</sup> 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.

# Weekend-free feeding with Gibco PSC Media

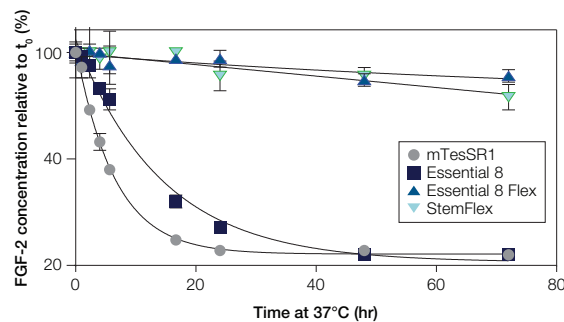
## Eliminate daily feeding schedules with confidence

Traditional methods of culturing PSCs require that the cultures be fed daily due to the heat sensitivity of key factors such as FGF2. Typically, the occasional weekend off is allowed by adjusting the protocol and hoping there's 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've created two unique formulations, Gibco™ Essential 8 Flex Medium and StemFlex Medium.

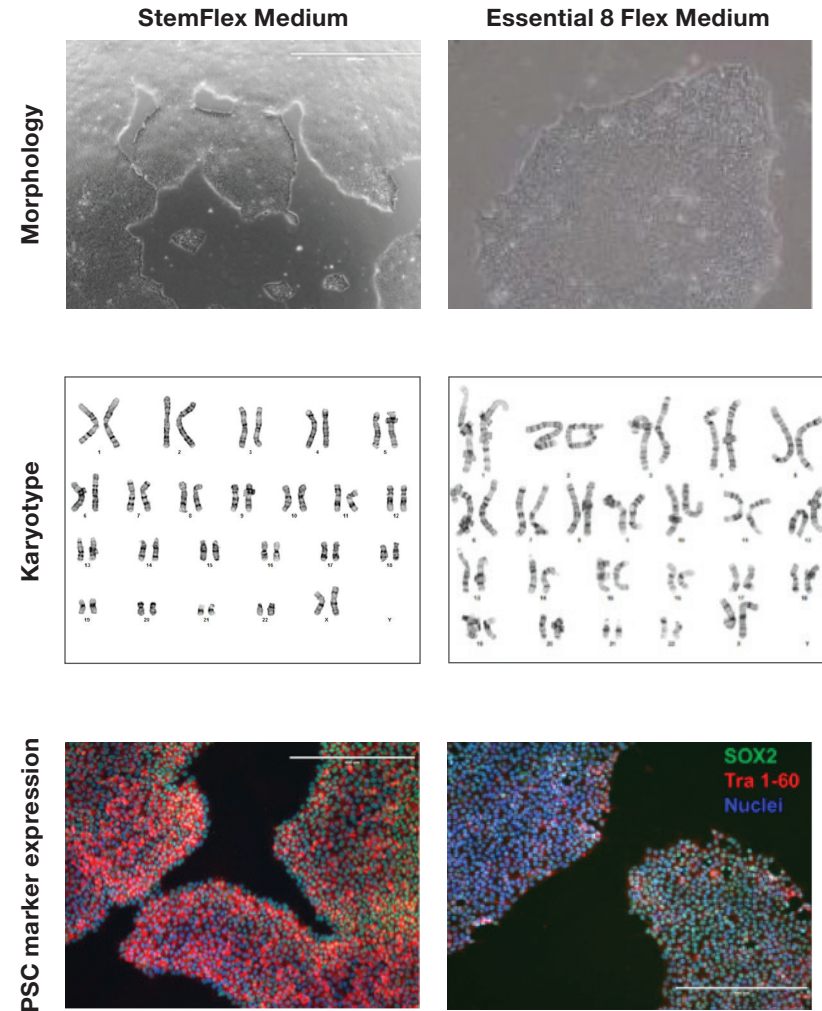
### Essential 8 Flex and StemFlex media:

- Contain wild type FGF2
- Maintain pluripotency more consistently by stabilizing heat sensitive components like FGF2 up to 50 passages (Figure 4)
- Allow for skipping up to 2 consecutive days for a total of 3 'feeding-free' days in a week (Figure 6)
- Reduce media consumption by up to 30% and thus costs compared to traditional feeder-free media

To learn more about these media visit [thermofisher.com/stemflex](https://thermofisher.com/stemflex) and [thermofisher.com/essential8flex](https://thermofisher.com/essential8flex)



**Figure 4. StemFlex and Essential 8 Flex media more consistently maintain pluripotency.** Both StemFlex and Essential 8 Flex Medium provide prolonged FGF-2 stability when incubated at 37°C, 5% CO<sub>2</sub>, allowing for flexible feeding schedules, including the weekend-free option—eliminating daily feeding requirements.



**Figure 5. Long-term maintenance of pluripotency in weekend-free feeding schedules.** PSCs exhibit normal morphology, karyotype, and expression of pluripotent stem cell markers following 50 passages in StemFlex medium on a Gibco™ Geltrex™ matrix (right) and in Essential 8 Flex medium on a Gibco™ Vitronectin™ matrix (left).

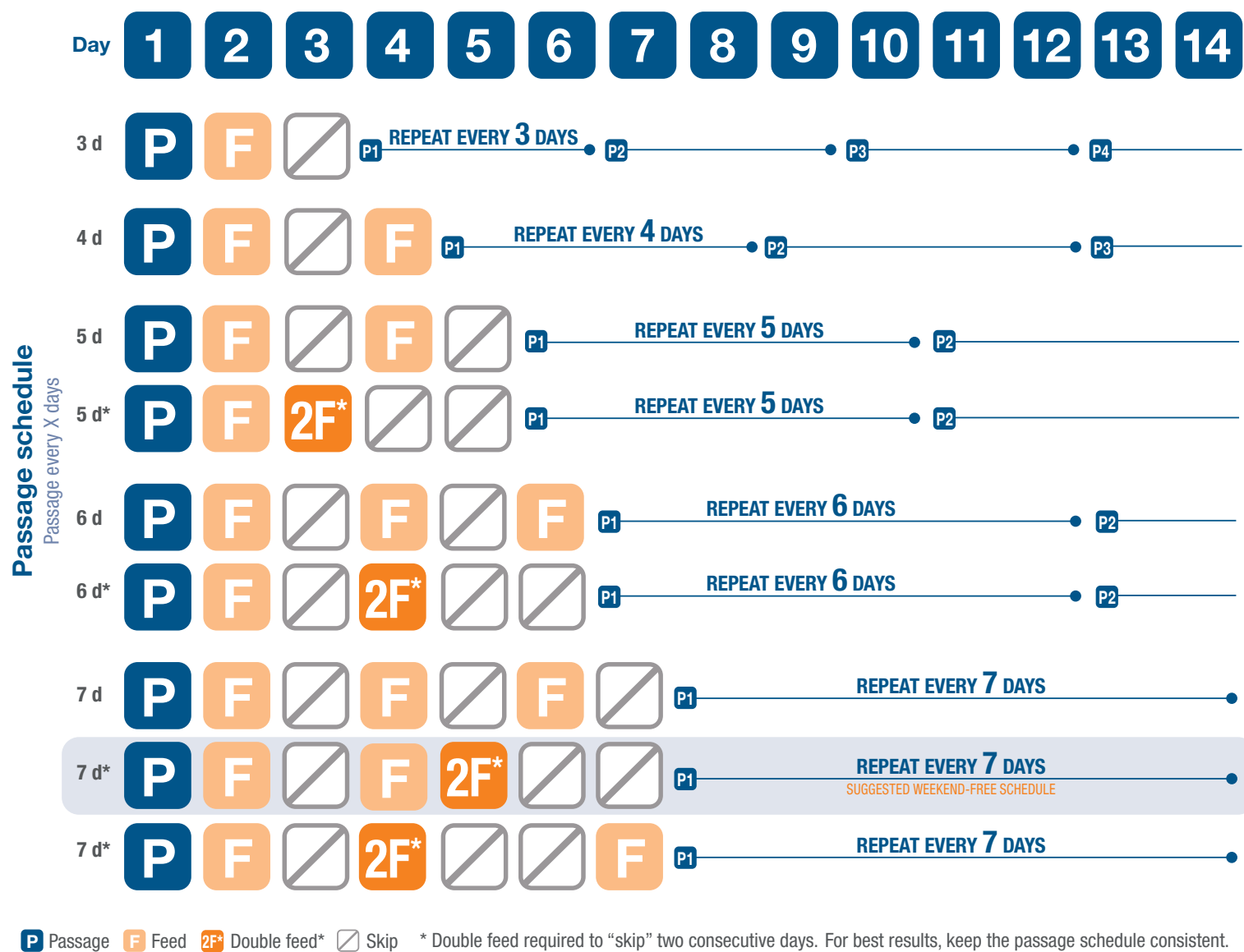


Figure 6. Alternative feed schedules for StemFlex and Essential 8 Flex Medium.

# KnockOut Serum Replacement – Multi-Species

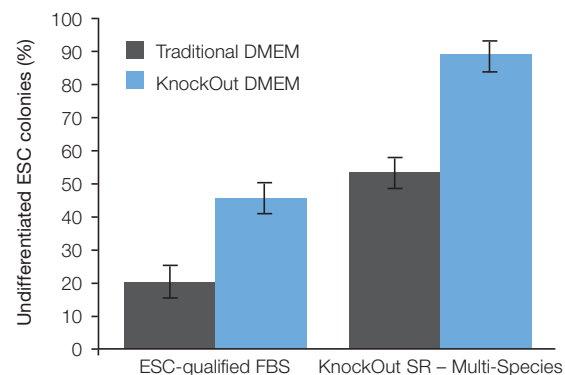
## Feeder-dependent culture proven more reliable than FBS

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.

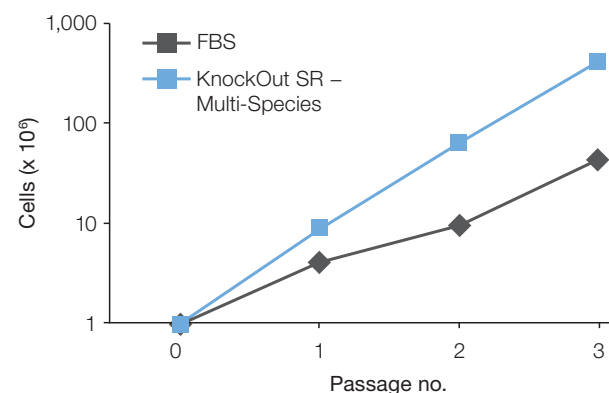
Gibco™ KnockOut™ Serum Replacement – Multi-Species (KnockOut SR – Multi-Species) is a more defined, FBS-free culture supplement designed to replace FBS in feeder-based PSC cultures. KnockOut SR – Multi-Species has been proven more reliable than FBS in mouse PSC and human PSC culture. It offers better 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](https://thermofisher.com/ksrmultispecies)



**Figure 7. Mouse PSC culture with KnockOut SR – Multi-Species vs. FBS in the absence of 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 8. Human PSC growth in KnockOut SR – Multi-Species vs. FBS.** H9 human ESCs were cultured on 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](https://thermofisher.com/cryopreservation)

**Table 7. Cryopreservation product overview.**

Product	PSC Cryopreservation Kit	Synth-a-Freeze Cryopreservation Medium	Recovery Cell Culture Freezing Medium
<b>Application</b>	Cryopreservation medium and recovery supplement optimized for maximum viability of PSCs	For freezing and storing a variety of cell types	Complete freezing medium for cryopreservation of a wide variety of mammalian cells
<b>Tested cell types</b>	iPSCs, ESCs, PBMCs, iPSC-derived cardiomyocytes	Human keratinocytes, MSCs, NSCs, other primary cell types	CHO-S, CHO-K1, HEK 293, Jurkat, NIH 3T3
<b>Chemical composition</b>	Xeno-free cryomedium; animal origin-free, chemically defined recovery supplement	Animal origin-free	Contains FBS
<b>Ready to use</b>	Yes	Yes	Yes
<b>Recovery component included</b>	Yes	No	No
<b>CTS product available</b>	N/A	CTS Synth-a-Freeze Cryopreservation Medium	N/A

# Characterization tools for PSC culture

Whether you are performing basic or more advanced characterization, validation is always critical in iPSC research. From Alkaline Phosphatase Live Stain, which provides quick verification of pluripotency, to the Applied Biosystems™ TaqMan® hPSC Scorecard™ Panel, which confirms trilineage differentiation potential, we have the tools you need to characterize your cells with confidence.

Visit [thermofisher.com/characterization](https://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	Efficient, easy-to-use characterization of undifferentiated stem cells	Evaluates pluripotency and confirms trilineage differentiation potential
<b>Product name</b>	Alkaline Phosphatase Live Stain	Antibody staining	TaqMan Human Stem Cell Pluripotency Array	TaqMan hPSC Scorecard Panel
<b>How specific are the results?</b>	Low (stains mouse and human stem and progenitor cells)	Medium (stains human ES and iPS cells)	Medium (profiles expression of human and mouse PSCs and tissue markers)	High (profiles expression of human PSCs and early germ layer markers)
<b>Will the cells remain viable?</b>	Yes	No	No	No
<b>How long before I see results?</b>	Stain PSCs typically in 20 minutes or less	Stain PSCs typically in 90–120 minutes	4–6 hours	6–8 hours
<b>Are data analysis tools included?</b>	No	No	No	Yes, free cloud-based software
<b>Is a reference standard included?</b>	No	No	No	Yes
<b>Are EVOS cell imager protocols available?</b>	Yes	Yes	N/A	N/A



## Need help characterizing your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals. See page 45 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].

Visit [thermofisher.com/scorecard](https://thermofisher.com/scorecard) to learn more about this innovative technology.

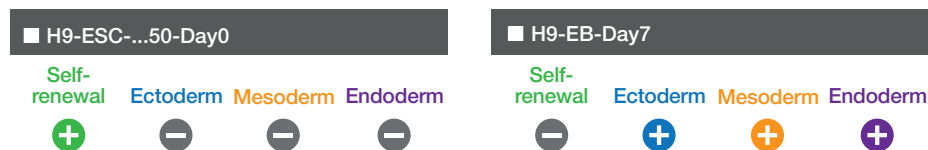


Figure 9. Gene expression results for self-renewal and germ layer markers are summarized in an easy-to-read format.

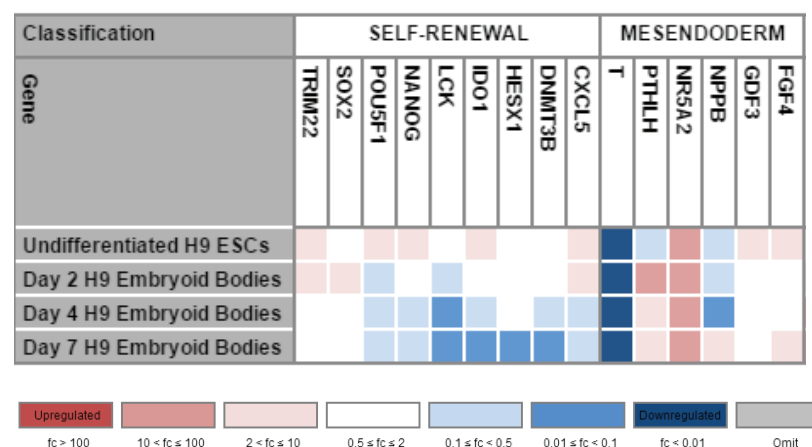


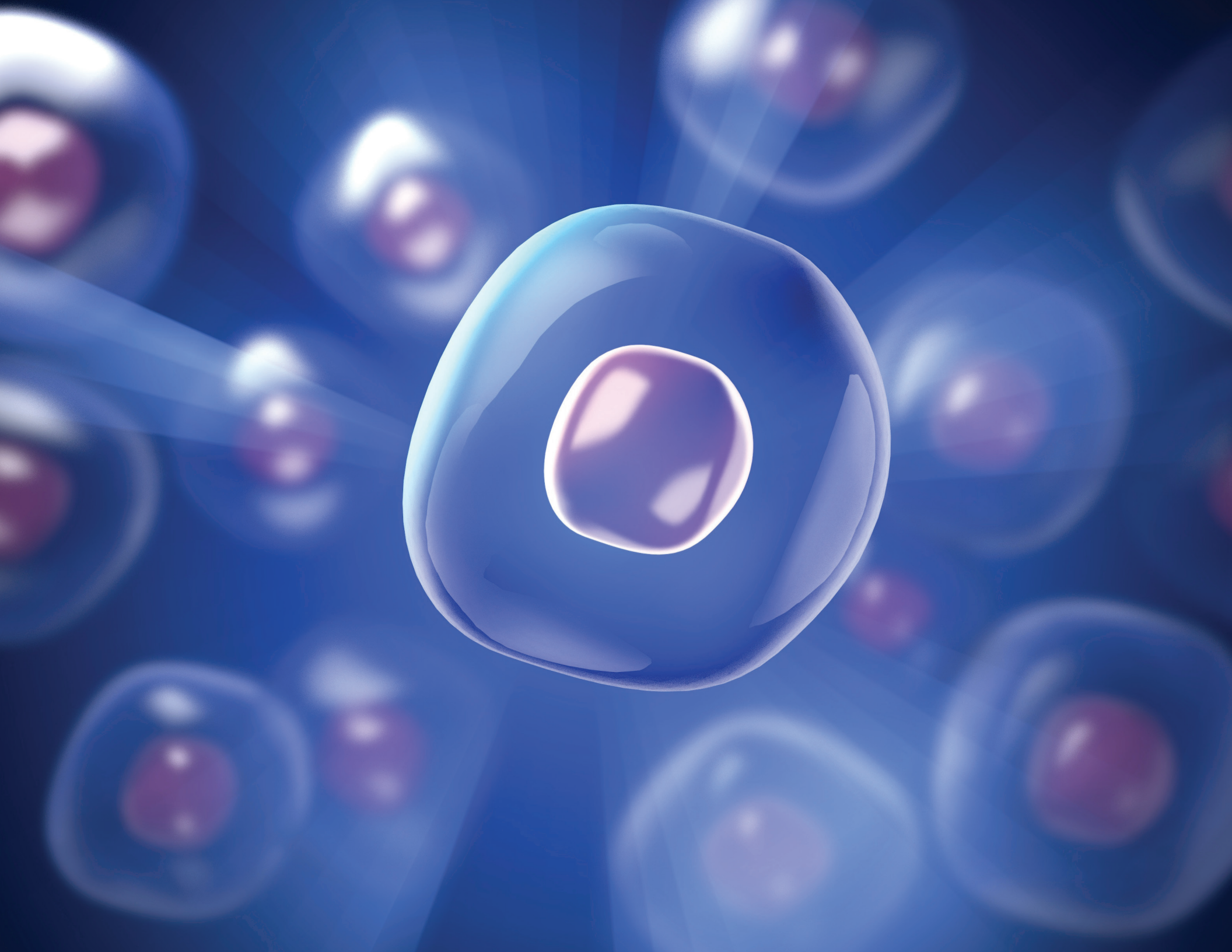
Figure 10. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.

## Invitrogen PSC antibody kits

### Superior imaging for PSCs in one box

The tools you need for superior image-based analysis of hPSCs and a variety of lineages are now available in one box. Kits include a combination of antibodies, stains, buffers, and/or media to create beautiful, high-quality images of stem cells.

Learn more about these and additional staining kits at [thermofisher.com/psccimmunokits](https://thermofisher.com/psccimmunokits)




































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](https://thermofisher.com/transfection)

## SUPPORT RESOURCES:

- View transfection protocols at [thermofisher.com/transfectionprotocols](https://thermofisher.com/transfectionprotocols)
- Download your copy of our transfection handbook at [thermofisher.com/transfectionhandbook](https://thermofisher.com/transfectionhandbook)

**Table 9. Transfection product selection guide—more blocks represent higher transfection efficiency into a great number of cell types.**

Transfection product	DNA	mRNA	RNAi	Protein	Co-delivery*	Primary cells	Stem cells	Suspension cells
<b>Superior transfection reagents</b>								
Lipofectamine 3000 Transfection Reagent								
Lipofectamine RNAiMAX Transfection Reagent								
Lipofectamine MessengerMAX Transfection Reagent								
<b>Electroporation</b>								
Neon Transfection System								
<b>CRISPR gene editing</b>								
Lipofectamine 3000 Transfection Reagent								
Lipofectamine MessengerMAX Transfection Reagent						Not yet tested		
Lipofectamine CRISPRMAX Cas9 Transfection Reagent						Not yet tested		

\* Cotransfection of RNAi vector and siRNA.

# Lipofectamine 3000 Transfection Reagent

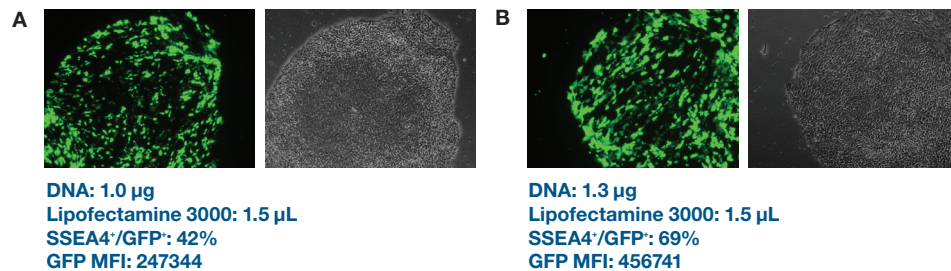
## Achieve over 70% transfection efficiency in difficult cells for just pennies per reaction

Invitrogen™ Lipofectamine™ 3000 Transfection Reagent was developed as a highly efficient, cost-effective nucleic acid delivery alternative to electroporation for stem cells. 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:

- Superior efficiency—10-fold higher efficiency into the broadest spectrum of difficult-to-transfect cells (Figure 11)
- Low toxicity—gentle on cells for improved viability
- Best value—just pennies per reaction for best-in-class transfection results\*

Learn more at [thermofisher.com/3000](https://thermofisher.com/3000)



**Figure 11. 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<sup>+</sup> cells.

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

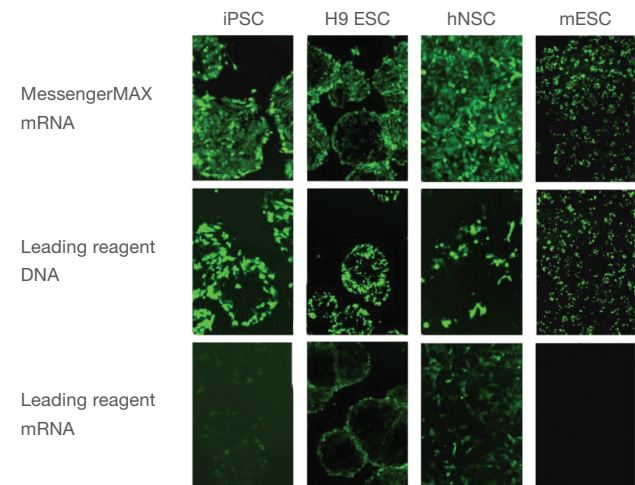
# Lipofectamine MessengerMAX Transfection Reagent

## The highest transfection efficiency in stem cells, primary cells, and neurons

Invitrogen™ Lipofectamine™ MessengerMAX™ mRNA 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 mRNA CRISPRs
- Direct delivery to cytoplasm—great for slow-dividing cells



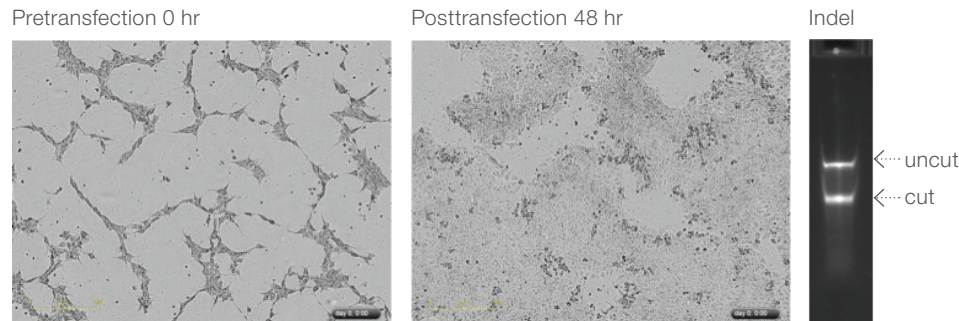
**Figure 12. Lipofectamine MessengerMAX reagent outperforms leading DNA delivery reagent and leading mRNA delivery reagent in various stem cells (Gibco™ iPSCs, H9 ESCs, mESCs, and hNSCs).** Lipofectamine MessengerMAX and the leading mRNA delivery reagent were used to deliver GFP mRNA (250 ng/well) in 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.

# Lipofectamine CRISPRMAX Transfection Reagent

## Simplicity of a reagent with effectiveness of electroporation for Cas9 protein delivery in genome editing

Invitrogen™ Lipofectamine™ CRISPRMAX™ Reagent is the first optimized transfection reagent for CRISPR-Cas9 protein delivery, providing up to 85% cleavage efficiency.

- Exceptional cleavage efficiency—demonstrated cleavage efficiency in over 20 cell types, including hiPSCs and mESCs
- Low cell toxicity—fewer cells needed to initiate your experiment
- Cost savings vs. electroporation—whether calculated as cost-per-reaction or initial investment
- Easily scalable—ideal delivery solution for high-throughput experiments



Cell type	Seeding cells		Locus	% indel
	Cell numbers/ well	% confluence (time of transfection)		
hiPSC	0.4 x 10 <sup>5</sup>	30%	HPRT1	55 ± 5%

# Neon Transfection System

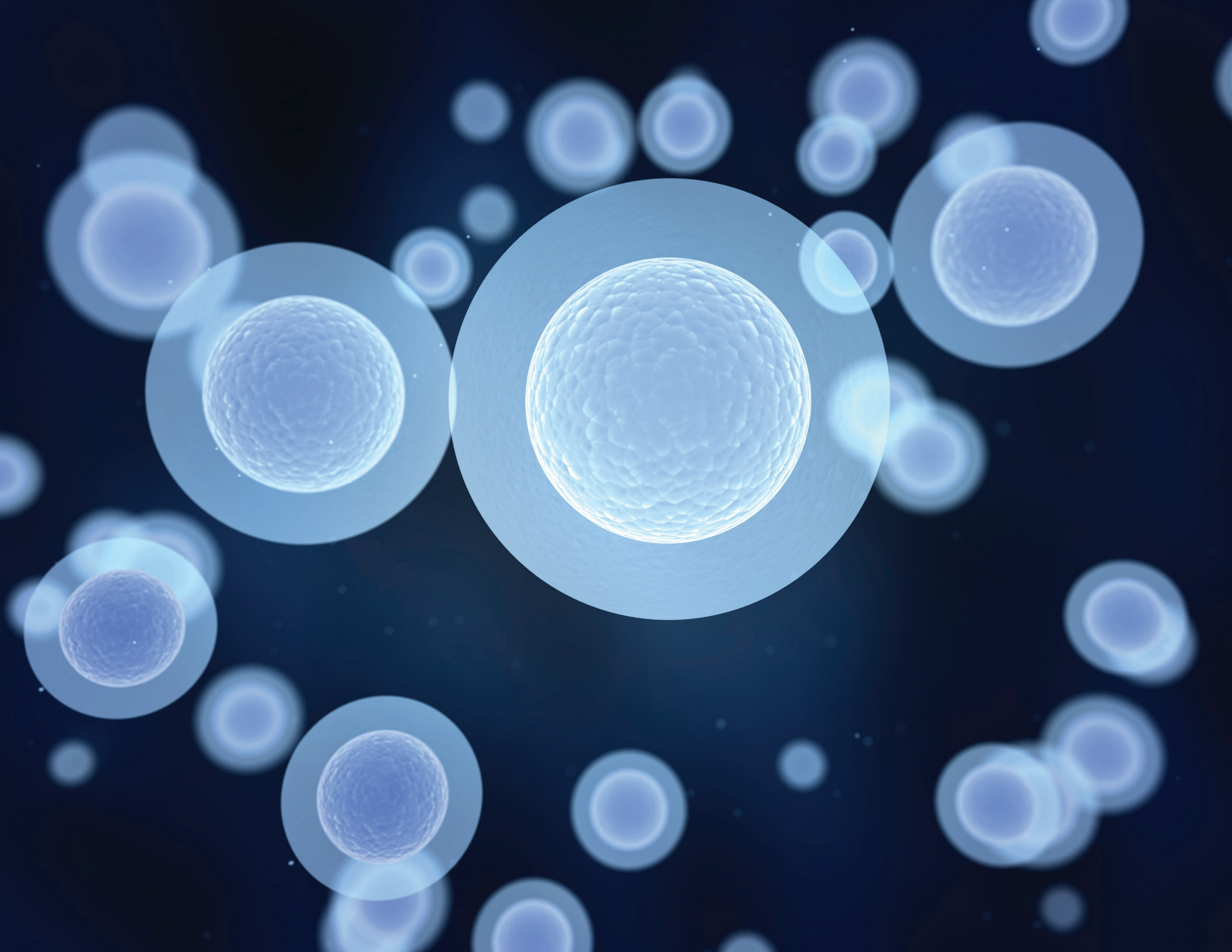
## Simple, customizable, and gentle electroporation instrument that delivers high transfection and cleavage efficiency

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 55% cleavage efficiency in stem cells
- Gentle—adjustable parameters enable more gentle transfection and higher cell viability
- Customizable—preprogrammed 24-well optimization protocols and open platform for additional protocols
- Compatible—use with StemFlex Medium during genome editing via electroporation

Cell line	Source	% indel	
		Lipofectamine CRISPRMAX Reagent	Neon electroporation
mESC	Mouse embryonic stem cell	75 ± 3	74 ± 4
hiPSC	Human episomal- induced pluripotent stem cell	55 ± 3	85 ± 2









Genome editing technologies, such as CRISPR and TAL effectors, 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.

Learn more about our genome editing products and services at [thermofisher.com/genomeedit](https://thermofisher.com/genomeedit)

## SUPPORT RESOURCES:

- New to genome editing? Access our 24–7 learning center at [thermofisher.com/genomeedit101](https://thermofisher.com/genomeedit101)
- Join our new hands-on CRISPR workshop; find out more at [thermofisher.com/CRISPRworkshop](https://thermofisher.com/CRISPRworkshop)
- Download the Genome Editing Resource Guide [thermofisher.com/genomeeditresourceguide](https://thermofisher.com/genomeeditresourceguide)

**Table 10. Gene 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	<ul style="list-style-type: none"><li>• Superior cleavage efficiency</li><li>• Simple design and assembly process</li><li>• Multiplexing capable</li></ul>	<ul style="list-style-type: none"><li>• Flexible; no sequence restriction or PAM requirement; ideal for knock-in</li><li>• Includes the rights under foundational TAL IP</li></ul>	<ul style="list-style-type: none"><li>• Ultimate flexibility in technology and gene targets</li><li>• High potency</li><li>• Minimal off-target effects</li></ul>	<ul style="list-style-type: none"><li>• Superior cleavage efficiency</li><li>• No cell-specific promoter constraint</li><li>• No random integration concern</li></ul>
Design requirement	PAM site (NGG)	Completely flexible, no design restrictions	NA	PAM site (NGG)
Ideal products for PSC	GeneArt Platinum Cas9 Nuclease and TRUEGuide sgRNA	GeneArt TAL effectors	Silencer Select siRNA Libraries	LentiArray CRISPR Libraries or Custom LentiPool CRISPR Libraries
Format	N/A	N/A	Array or pooled	Array or pooled



## Need help engineering your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals. See page 45 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](https://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/cellineservice](https://thermofisher.com/cellineservice)

**Table 11. Available CRISPR-Cas9 delivery formats.**

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

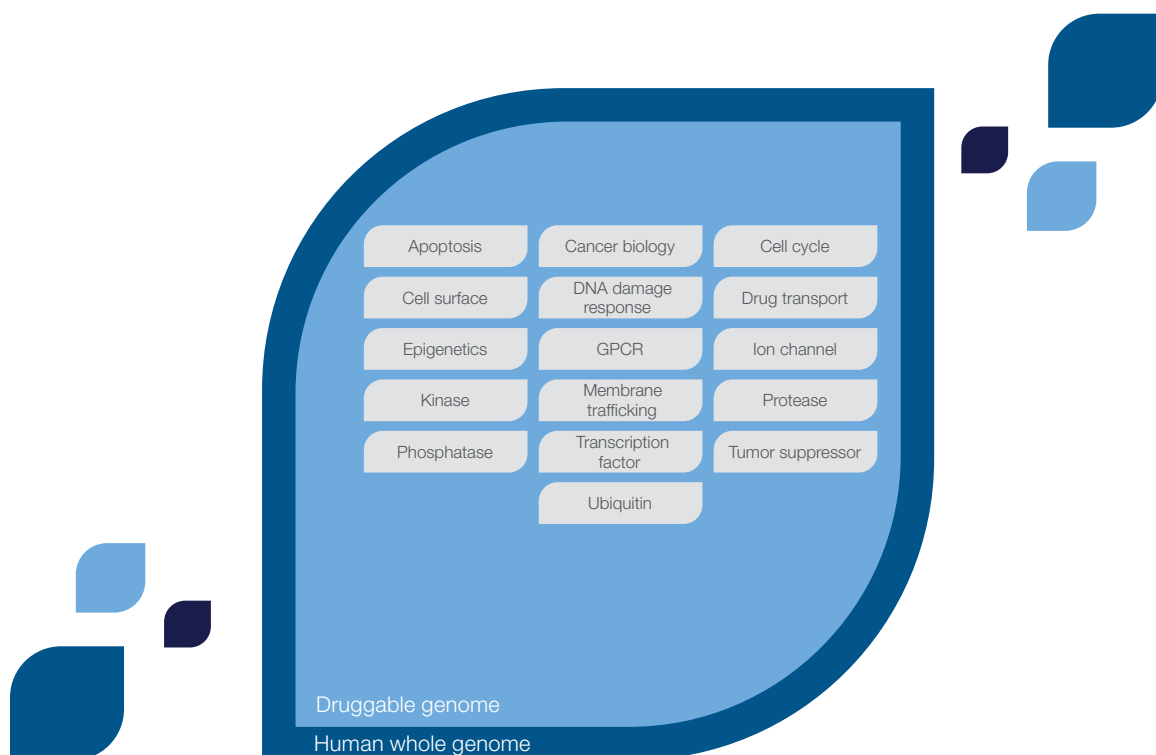
\* Awarded Top 10 Innovations in 2016 by *The Scientist* Magazine.

# 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](https://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 cell line.

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

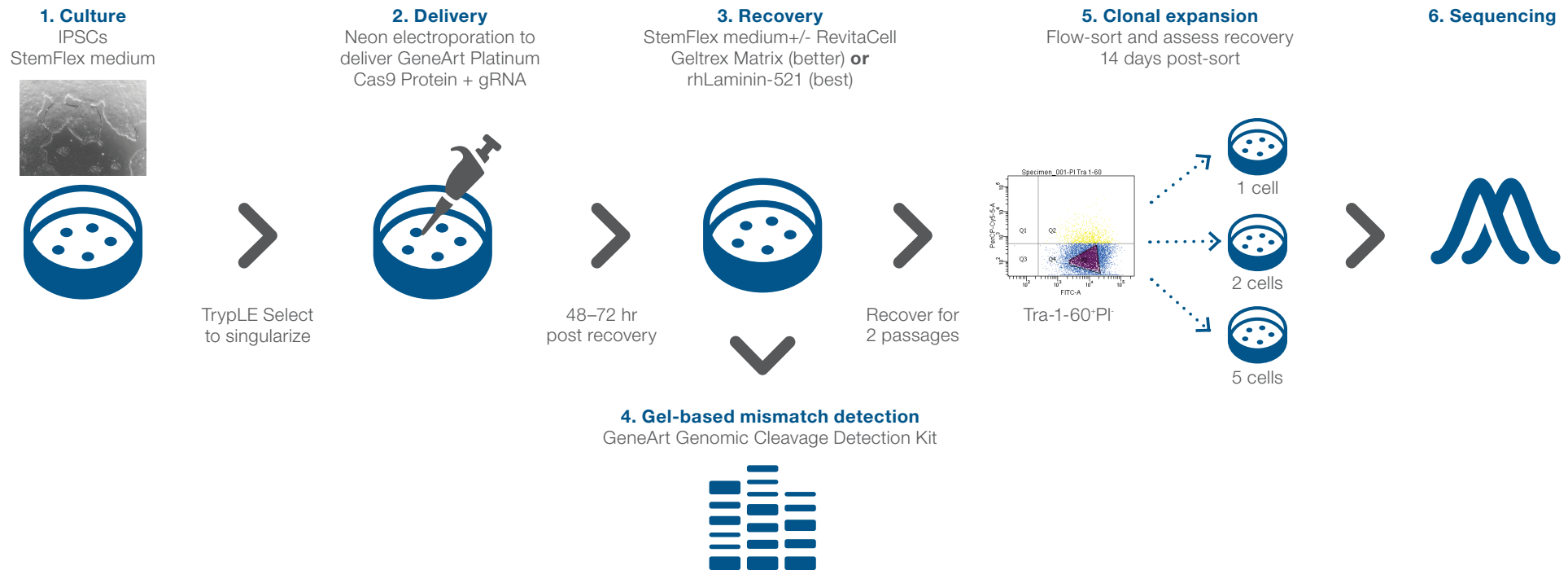
- Performance: >30% cleavage in most target loci tested
- Quality: Extensive characterization to confirm karyotype, pluripotency potential, and genome editing efficiency
- Flexibility: Ability to differentiate into your desired terminal cell type following editing

To gain access to the Human Episomal Cas9 iPSC cell line, submit an inquiry at [thermofisher.com/askdiscovery](https://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.

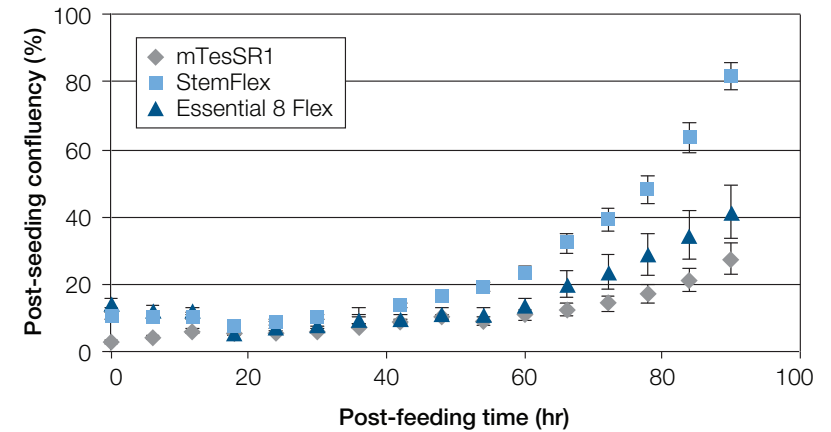


**Figure 13. Standard gene editing workflow using CRISPR-Cas9 technology.** Following the expansion of hPSCs with the StemFlex/Geltrex media system, cells are singularized and electroporated using the Neon system to introduce precomplexed Cas9 protein and control gRNA. Cells recover in StemFlex medium in the presence or absence of RevitaCell supplement on either Geltrex substrate or Laminin521. Following 48–72 hours of recovery, cleavage efficiency is assessed using the 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. Following 14 days of recovery, successful clonal expansion is determined, followed by determination of successful gene editing of clonally established lines through sequencing.

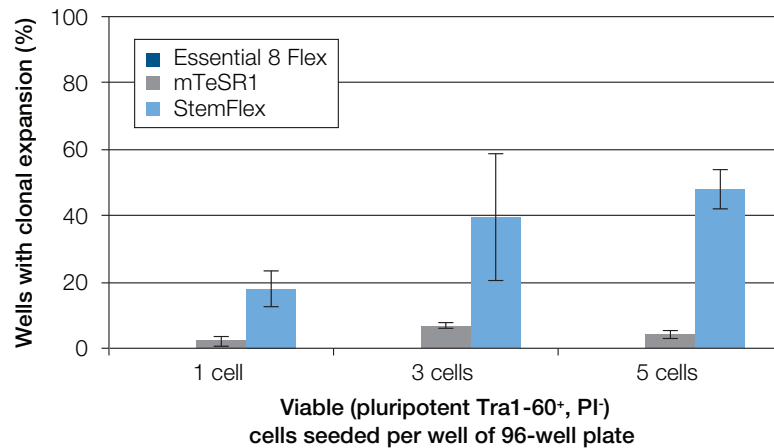


**Table 12. Comparison of plasmid DNA, Cas9 mRNA/gRNA, and Cas9 RNP transfection, and resulting editing efficiencies as measured by GCD assay in a variety of cell lines.**

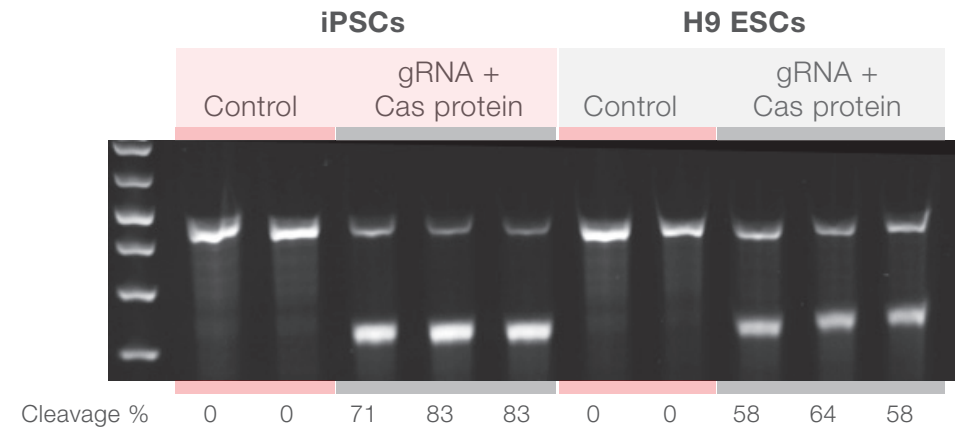
Cell lines	Plasmid		mRNA		Protein	
	Lipid	Electro.	Lipid	Electro.	Lipid	Electro.
HEK293FT	49	49	70	40	51	88
U2OS	15	50	21	24	18	70
Mouse ESCs	30	45	45	20	25	70
Human ESCs (H9)	0	8	20	50	0	64
Human iPSCs	0	20	66	32	5	87
N2A	66	75	66	80	66	82
Jurkat	0	63	0	42	0	94
K562	0	45	0	27	0	72
A549	15	44	23	29	20	66
Human Keratinocytes (NHEK)	0	30	0	50	0	35



**Figure 15. 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.

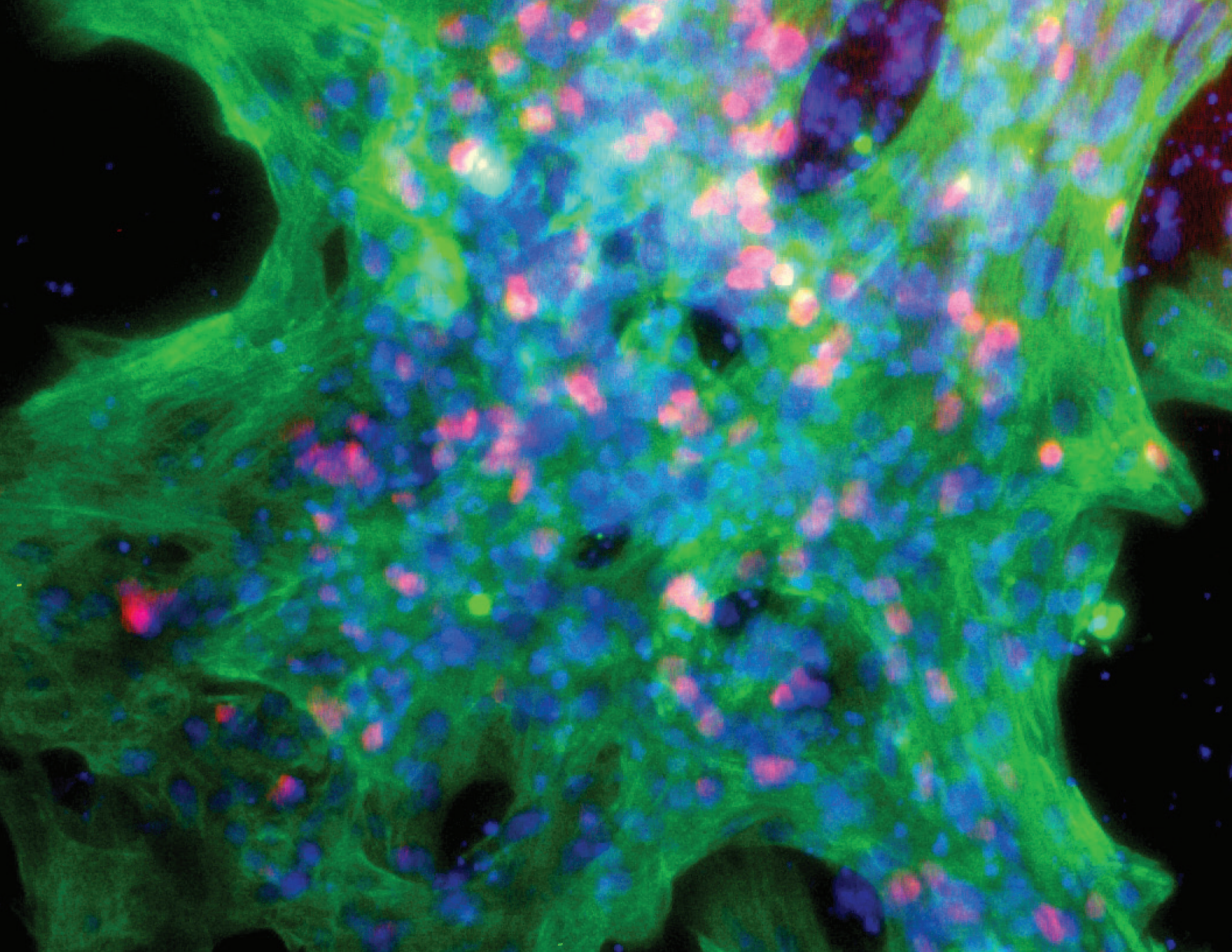


**Figure 14. Comparison of cell recovery following flow sorting.** Cells were evaluated in the three different media without the need for a ROCK inhibitor and plated at 1, 3, or 5 cells per well on rhLaminin-521. This data demonstrates that StemFlex Medium is the only system that enables significant clonal expansion when only a single cell is plated per well, even in the absence of RevitaCell (or a ROCK inhibitor).



**Figure 16. Genome editing of human stem cells with the GeneArt Cas9-gRNA RNP.** Gibco™ human iPSCs and H9 ESCs were transfected in triplicate with the Invitrogen™ GeneArt Cas9-gRNA ribonucleoprotein (RNP) complex, and target sites were analyzed for cleavage using the Invitrogen™ GeneArt Genomic Cleavage Detection (GCD) Kit 48–72 hours posttransfection.







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.

View the complete differentiation portfolio at [thermofisher.com/differentiation](https://thermofisher.com/differentiation)

SUPPORT RESOURCES:

- View differentiation protocols at [thermofisher.com/stemcellprotocols](https://thermofisher.com/stemcellprotocols)
- Request a copy of the Neurobiology Protocol Handbook at [thermofisher.com/neurohandbook](https://thermofisher.com/neurohandbook)

Table 13. 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 Supplement and Neurobasal Medium	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	N/A



Need help differentiating your cells?

We have a dedicated team of stem cell scientists to help you achieve your project goals. See page 45 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 17) 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 18). 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 19).

For more information, go to [thermofisher.com/nscdiff](https://thermofisher.com/nscdiff)

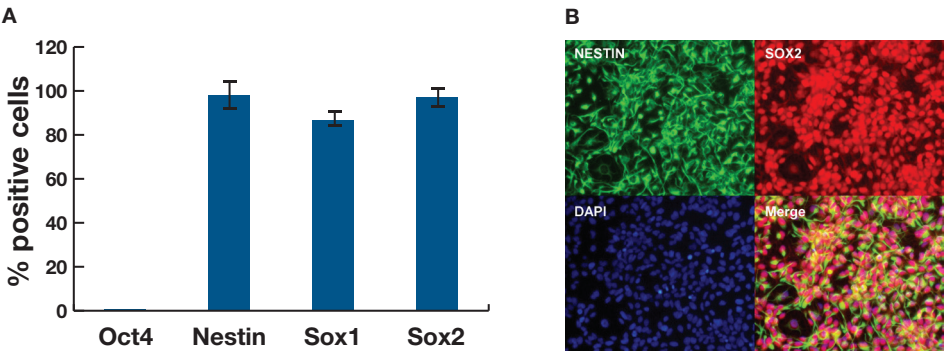


Figure 17. NSCs generated using PSC Neural Induction Medium express high levels of NSC markers Nestin, Sox1, and Sox2, and low levels of residual pluripotent marker Oct4. (A) 80–90% neural induction efficiency. (B) ICC staining images of relevant NSC markers.

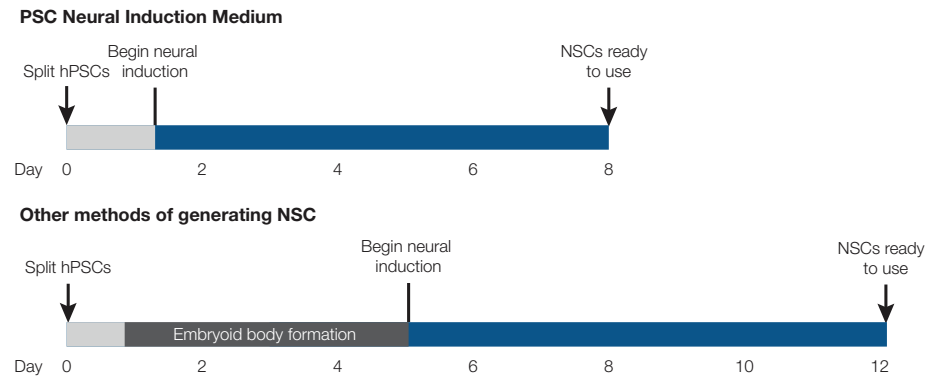


Figure 18. Unlike existing methodologies, PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation which adds time, labor, and variability.

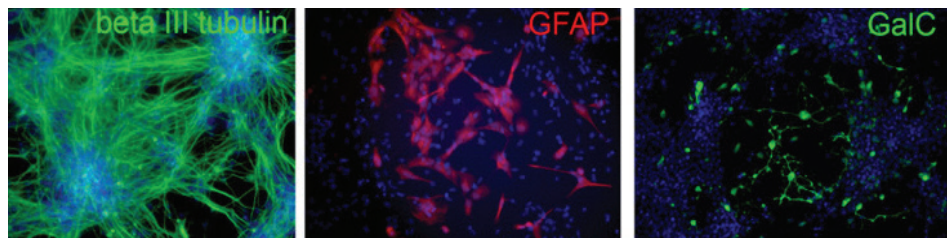


Figure 19. Neural stem cells (NSCs) generated using PSC Neural Induction Medium have high expression of NSC markers and can be further differentiated into other neural cell types.

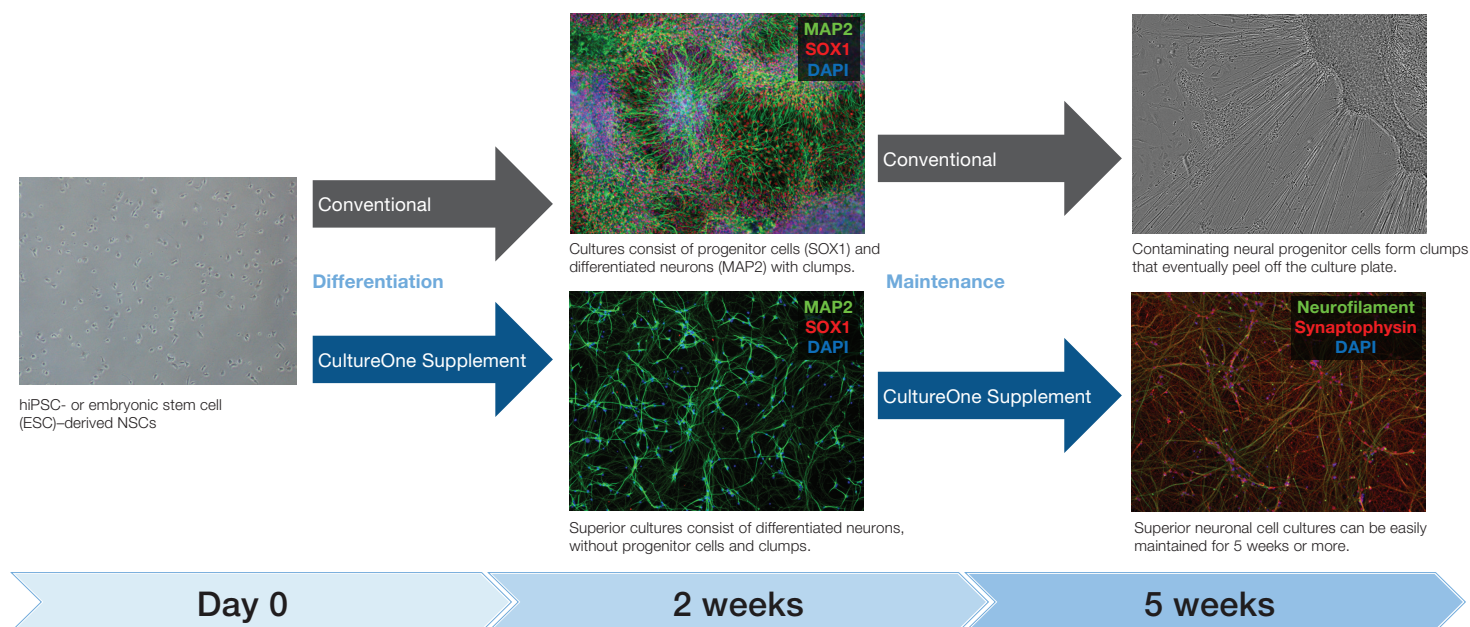


# CultureOne Supplement

## Superior neuronal cell cultures

Gibco™ CultureOne™ Supplement is a xeno-free supplement designed to significantly improve 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 20).

For more information, go to [thermofisher.com/cultureone](https://thermofisher.com/cultureone)



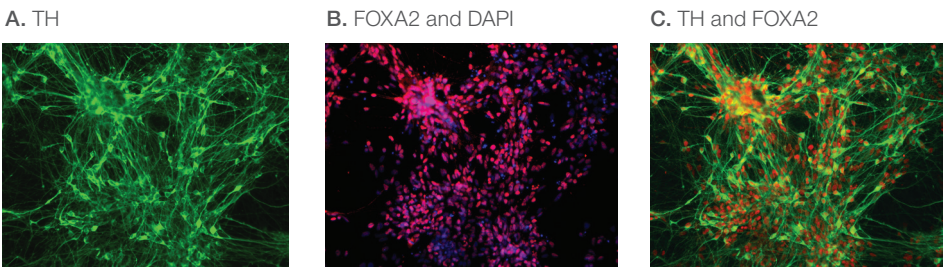
**Figure 20.** H9 ESC-derived NSCs were plated at density of  $5 \times 10^4$  cells/cm<sup>2</sup>. Without CultureOne Supplement, cells at 2 weeks of differentiation were highly dense, formed cell clumps, and contained MAP2 positive neurons and a significant number of SOX1-positive NSCs. At 2 weeks 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 MEA.

# PSC Dopaminergic Neuron Differentiation Kit

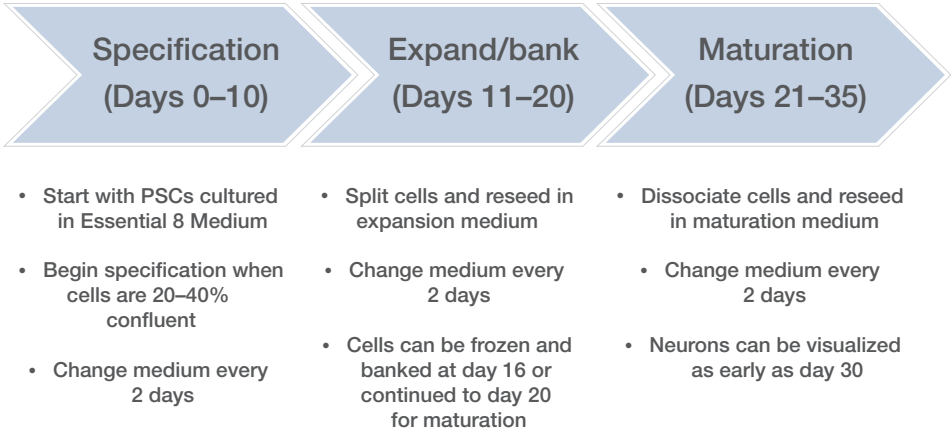
## Differentiate iPSCs to functional midbrain dopaminergic neurons

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 also has the ability to maintain a precursor population of cells which can be expanded and banked.

For more information, go to [thermofisher.com/dopadiff](https://thermofisher.com/dopadiff)



**Figure 21. 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).



**Figure 22. 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.

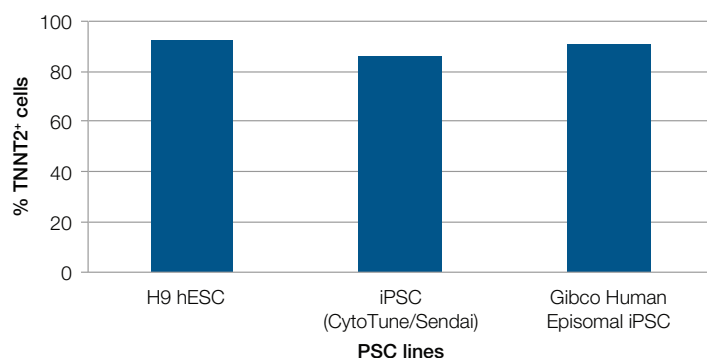
# PSC Cardiomyocyte Differentiation Kit

## Three simple steps. One simple kit.

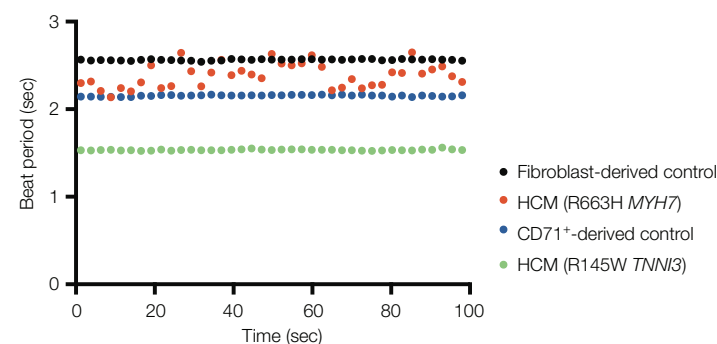
The Gibco™ PSC Cardiomyocyte Differentiation Kit consists of a set of serum-free and xeno-free media that enable efficient differentiation of human PSCs to contracting cardiomyocytes in as few as 8 days. Unlike other methods that require multiple components and longer assay duration, the PSC Cardiomyocyte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

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

Find out more at [thermofisher.com/cardiadiff](https://thermofisher.com/cardiadiff)



**Figure 23. 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



**Figure 24. 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.

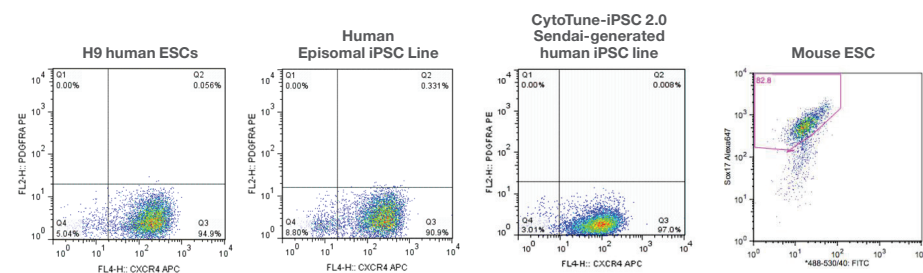
# PSC Definitive Endoderm Induction Kit

## Definitive endoderm cells in 48 hours

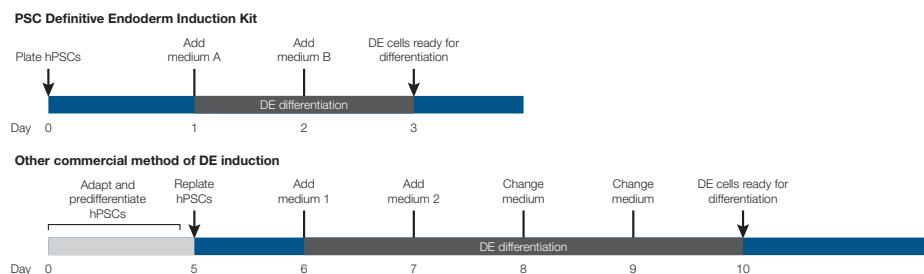
The Gibco™ PSC Definitive Endoderm Induction Kit consists of two xeno-free media that enable efficient induction of human pluripotent stem cells to definitive endoderm. Unlike other methods that require multiple components and take 5 or more days, the PSC Definitive Endoderm Induction Kit enables you to generate  $\geq 90\%$  CXCR4<sup>+</sup>/PDGFR $\alpha$ <sup>-</sup> definitive endoderm cells with only 2 components in just 2 days (Figure 26).

Each medium is supplied as a 1X complete medium, requiring no mixing of additional components, and the resultant definitive endoderm show more than 90% high expression of key markers SOX17 and FOXA2 across multiple PSC lines (Figure 25) and are capable of differentiating to downstream lineages.

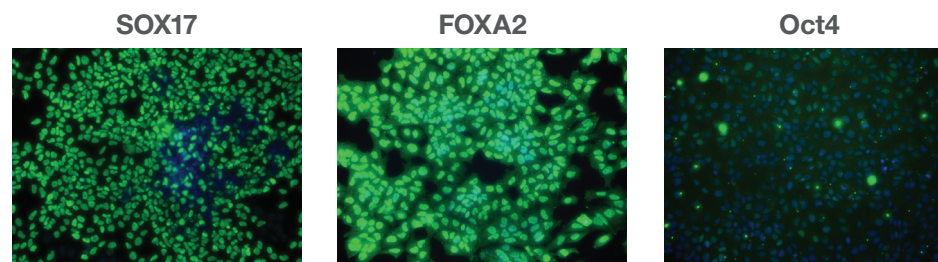
See the complete set of data at [thermofisher.com/dendo](https://thermofisher.com/dendo)



**Figure 25. 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<sup>+</sup>/PDGFR $\alpha$ <sup>-</sup> cell populations derived from various cell lines. Representative dot plot for mESCs shows a Sox17<sup>+</sup> cell population. For each experiment, unstained cells were used to set gates.



**Figure 26. 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 27. Immunocytochemistry of hESCs treated with the PSC Definitive Endoderm Induction Kit.** At day 3, induced cells were immunostained for the endodermal transcription factors SOX17 and FOXA2, and the pluripotent marker Oct4. Nuclei were counterstained with DAPI (blue) to assess total cell numbers.

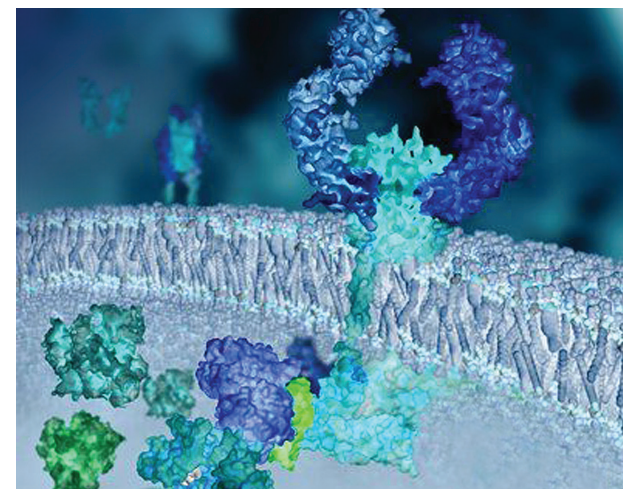




# Differentiation: growth factors

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 (Cell Therapy Systems) 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, FGF2)**

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 hematopoiesis, neural development, and inflammation.

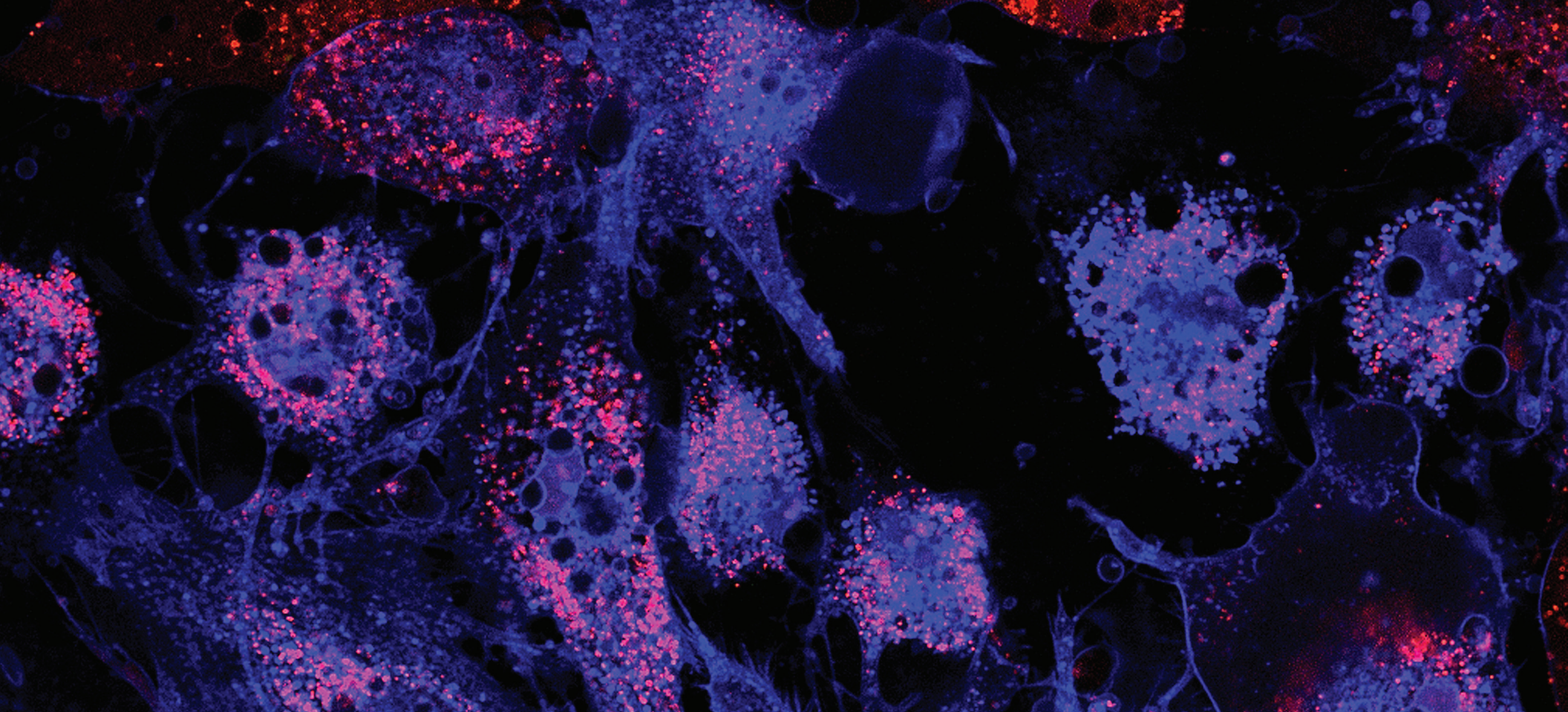
## **Tumor necrosis factor (TNF)**

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](https://thermofisher.com/growthfactors)



## Characterization and analysis tools

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

Choose among the tools and services for stem cell analysis at [thermofisher.com/stemcellanalysis](https://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
- Primary antibodies
- 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
- Western blotting kits

### Sample preparation

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

- Invitrogen™ TaqMan® PreAmp Cells-to-C<sub>T</sub>™ Kit
- Applied Biosystems™ protein expression sample preparation kits
- Invitrogen™ RNA extraction and purification kits
- Invitrogen™ DNA purification kits

### Genomic analysis

Trusted qRT-PCR and sequencing platforms for a wide variety of genomic analyses.

- Applied Biosystems™ TaqMan® Gene Expression Assays
- Applied Biosystems™ TaqMan® miRNA Assays
- Applied Biosystems™ TaqMan® SNP Assays
- Applied Biosystems™ TaqMan® CNV Assays
- Applied Biosystems™ AuthentiFiler™ PCR Amplification Kit
- Ion AmpliSeq™ panels



### Find your Ab match

With over 40,000 antibodies covering many stem cell targets, find the best antibody for your research. Find antibodies for all stem cell targets by visiting [thermofisher.com/antibodies](https://thermofisher.com/antibodies)

# Select 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](https://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. Learn more about the Countess II FL instrument at [thermofisher.com/countess](https://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. Learn more about the Attune NxT Flow Cytometer at [thermofisher.com/attune](https://thermofisher.com/attune)



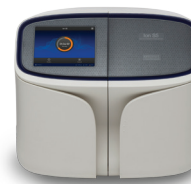
## StepOnePlus Real-Time PCR System

The Applied Biosystems™ StepOnePlus™ Real-Time PCR System includes additional performance features supporting the full range of TaqMan assays, while providing ease of use and a small footprint. The StepOnePlus Real-Time PCR System enables an easy-to-use molecular assessment of pluripotent stem cells. Go to [thermofisher.com/steponeplus](https://thermofisher.com/steponeplus) to watch the StepOnePlus video.



### 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](https://thermofisher.com/quantstudio)



### Ion S5 and S5 XL Systems

Adopting next-generation sequencing (NGS) is now simpler than ever. The Ion S5™ and Ion S5™ XL Systems enable the simplest targeted sequencing workflow with industry-leading speed and affordability. See how at [thermofisher.com/ions5](https://thermofisher.com/ions5)



### Ion Personal Genome Machine (PGM) sequencer

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 next-generation sequencer. Go to [thermofisher.com/pgm](https://thermofisher.com/pgm) to learn more about the Ion PGM™ System.





# 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](https://thermofisher.com/ctsstemcells)

## SUPPORT RESOURCES:

- Download the cell therapy solutions brochure at [thermofisher.com/celltherapysolutions](https://thermofisher.com/celltherapysolutions)
- Access the CTS Mini-documentary series videos at [thermofisher.com/cts-videoseries](https://thermofisher.com/cts-videoseries)



### cGMP-compliant manufacturing

- Manufactured in conformity with cGMP for medical devices, 21 CFR Part 820 of the regulation
- US Food and Drug Administration (FDA)-registered manufacturing site with an ISO 13485-certified quality management system



### Testing and documentation

- Traceability documentation—including Certificates of Analysis, Certificates of Origin, and Drug Master Files
- Extensive QC testing for sterility, endotoxins, adventitious agents, and mycoplasmas on most products

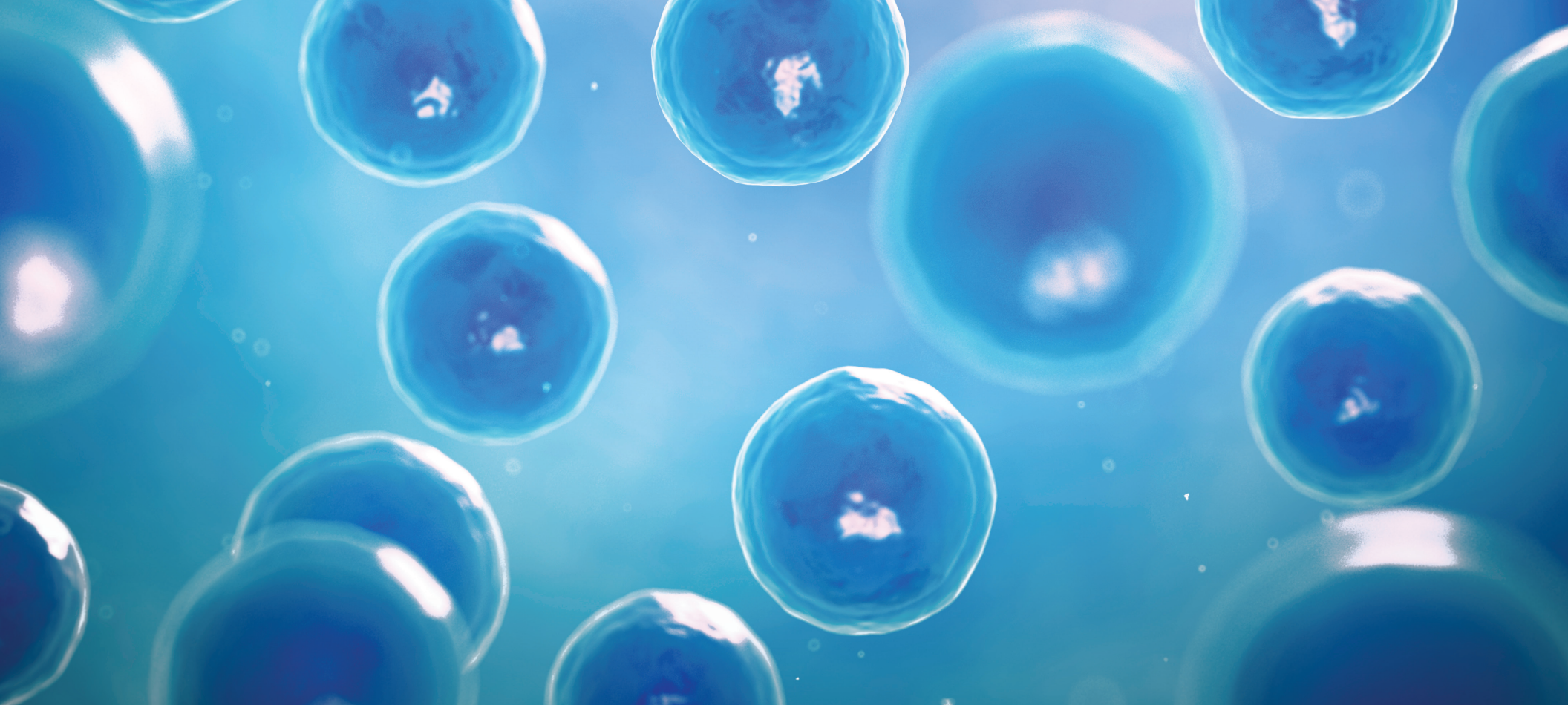


### Expert support

- Knowledgeable regulatory support team will help you navigate regulatory processes from research through commercialization
- Experienced cell therapy professionals available to help answer your questions

# Pluripotent stem cell therapy workflow solutions





## CellModel Services

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.



To inquire about other services or instrumentation, visit [thermofisher.com/askdiscovery](https://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](https://thermofisher.com/cellmodels)

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

- Dedicated team of stem cell scientists to deliver on your project
- Detailed protocols provided to you after project completion to demonstrate how we reached each milestone and document which 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**  
Sr. Manager  
Custom Biology R&D

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

We can take cell-based or stem cell-based assays and configure not just large-scale provisioning of these cells, but we can transfer them directly into a 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:

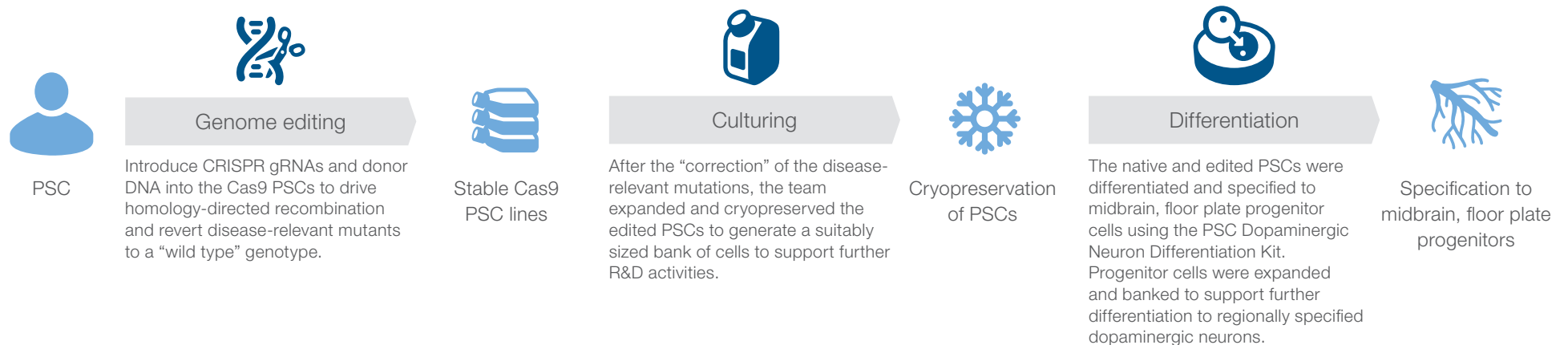
“The services staff had a high level of expertise and a genuine interest in making sure that the project was successful. All personnel were highly knowledgeable and professional. My initial meetings and discussions set a very positive tone for the services and professionalism of Thermo Fisher Scientific.”

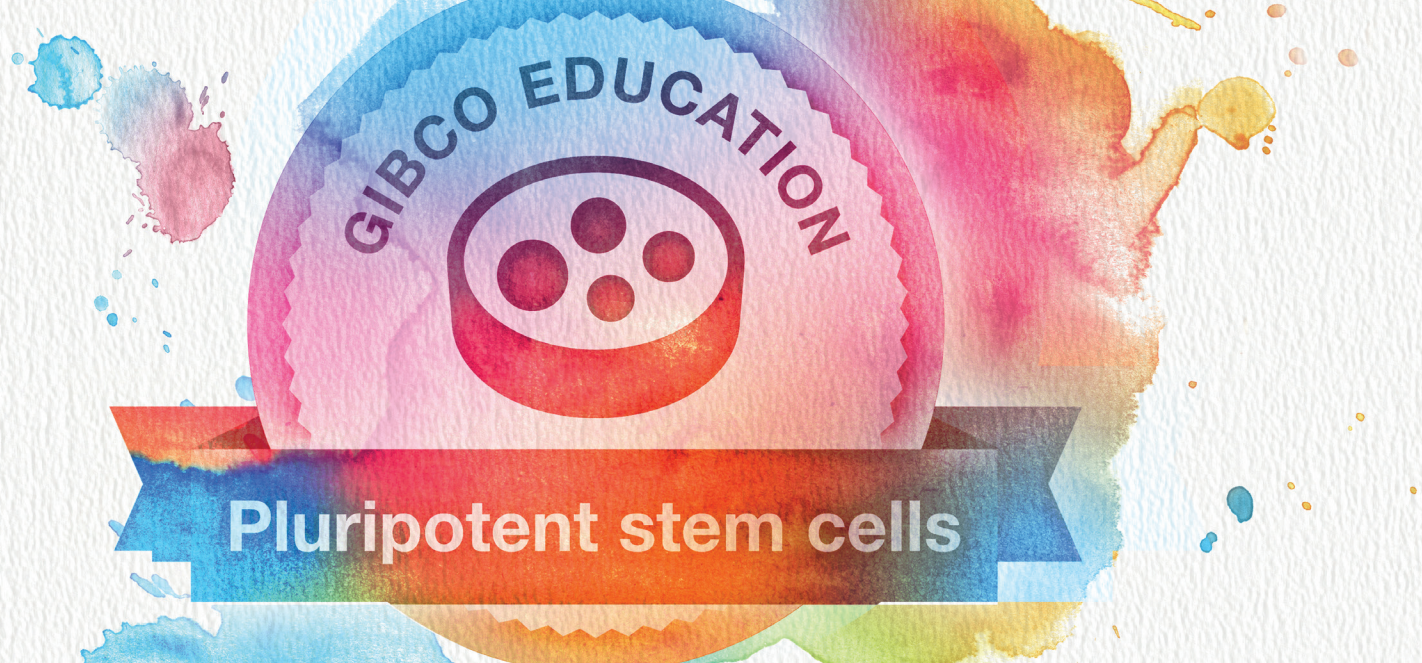
“Our request was well organized, price points well explained, and shipped to us at a convenient time, avoiding the holiday period. The team was accommodating when we were unsure of our own MTA arrangements.”

“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.





## 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](https://thermofisher.com/psceducation)



### Expand your knowledge with:

- Key assets such as our Pluripotent Stem Cell 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, CA, Frederick, MD, and Glasgow, 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](https://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 comprised of both lectures and hands-on laboratory work at our state-of-the-art training facility.

Learn more or register today at [thermofisher.com/crisprworkshop](https://thermofisher.com/crisprworkshop)



## Ordering information

Product	Cat. No.
<b>Somatic and progenitor cells</b>	
CTS Immune Cell Serum Replacement*	A25961-01
Human Dermal Fibroblasts, Adult	C-013-5C
StemPro BM Mesenchymal Stem Cells	A15652
StemPro CD34 <sup>+</sup> 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
StemPro-34 SFM	10639-011
<b>Reprogramming</b>	
CytoTune-iPS 2.0 Sendai Reprogramming Kit (1 pack)	A16517
CytoTune-iPS 2.0 Sendai Reprogramming Kit (3 pack)	A16518
CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit (1 pack)	A34546
Epi5 Episomal iPSC Reprogramming Kit	A15960
Episomal iPSC Reprogramming Vectors	A14703
<b>Transfection</b>	
ExpiFectamine 293 Transfection Kit	A14526
InvivoFectamine 3.0 Reagent	IVF3001
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	CMAX00015
Lipofectamine 3000 Transfection Reagent	L3000-001
Lipofectamine MessengerMAX Transfection Reagent	LMRNA015
Lipofectamine RNAiMAX Transfection Reagent	13778-075
<b>Genome editing</b>	
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	B25642
GeneArt Platinum Cas9 Nuclease (1 µg/µL)	B25640

Product	Cat. No.
GeneArt Platinum Cas9 Nuclease (3 µg/µL)	B25641
GeneArt CRISPR Nuclease mRNA	A29378
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit	A21174
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 (with competent cells)	A21178
LentiArray Cas9 Lentivirus	A32064
LentiArray Cas9 Lentivirus	A32069
GeneArt Precision gRNA Synthesis Kit	A29377
TRUEGuide sgRNA	A32044
LentiArray Lentiviral sgRNA	A32042
LentiArray CRISPR Positive Control Lentivirus, human HPRT	A32056
LentiArray CRISPR Positive Control Lentivirus, human HPRT with GFP	A32060
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	A32062
LentiArray CRISPR Negative Control Lentivirus, human non-targeting with GFP	A32063
LentiArray CRISPR Negative Control Lentivirus, human non-targeting	A32327
GeneArt Genomic Cleavage Detection Kit	A24372
GeneArt Genomic Cleavage Selection Kit	A27663
Introduction to CRISPR-Cas9 Genome Editing Hands-on Workshop	A33133
Cas9 iPSC	Contact GEMServices@ thermofisher.com
Cas9 stable cell line	Contact GEMServices@ thermofisher.com



Product	Cat. No.
<b>Culture</b>	
Collagenase IV	17104-019
CTS TrypLE Select Enzyme <sup>†</sup>	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 <sup>**</sup>	10828-028
KnockOut Serum Replacement – Multi-Species	A31815-02
StemPro Accutase Cell Dissociation Reagent	A1110501
StemFlex Medium	A3349401
StemPro EZPassage Disposable Stem Cell Passaging Tool	23181-010
StemPro hESC SFM	A10007-01
TrypLE Express Enzyme (1X), no phenol red	12604013
<b>Matrices and feeder cells</b>	
CTS CELLstart Substrate <sup>†</sup>	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 Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903
Gibco CF1 Mouse Embryonic Fibroblasts, Irradiated	A34181
Gibco CF1 Mouse Embryonic Fibroblasts, MitC-Treated	A34959

Product	Cat. No.
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
rhLaminin-521	A29248
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
<b>Cryopreservation</b>	
CTS Synth-a-Freeze Cryopreservation Medium <sup>†</sup>	A13713-01
PSC Cryopreservation Kit	A2644601
Recovery Cell Culture Freezing Medium	12648-010
RevitaCell Supplement	A26445-01
Synth-a-Freeze Cryopreservation Medium	A12542-01
<b>Differentiation</b>	
Activin A Recombinant Human Protein	PHC9564
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
PSC Definitive Endoderm Induction Kit	A3062601
PSC Dopaminergic Neuron Differentiation Kit	A3147701
PSC Neural Induction Medium	A1647801

Unless otherwise indicated, all products are For Research Use Only. For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. Caution: Not intended for direct administration into humans or animals.

Not for use in diagnostic procedures.

<sup>\*</sup> For *In Vitro* Diagnostic Use.

<sup>\*\*</sup> 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.

<sup>†</sup> For Research Use or Noncommercial Manufacturing of Cell-Based Products for Clinical Research. CAUTION: Not for direct administration into humans or animals.



Product	Cat. No.
TNF Recombinant Human Protein	PHC3015
VEGF Recombinant Human Protein	PHC9394
<b>Characterization</b>	
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
KLF4 Antibody	710659
LIN28 Antibody	MA1-016
NANOG Antibody	MA1-017
OCT4 Antibody	A13998
Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit	A24881

Product	Cat. No.
PSC Immunocytochemistry Kit (OCT4, SSEA4)	A25526
PSC Immunocytochemistry Kit (SOX2, TRA-1-60)	A25525
SALL4 Antibody	720030
SOX 2 Antibody	48-1400
SSEA-1 Antibody	MA1-022
SSEA-3 Antibody	MA1-020
SSEA-4 Antibody	MA1-021
SSEA-5 Antibody	MA1-144
TaqMan hPSC Scorecard Panel, 384-well	A15870
TaqMan hPSC Scorecard Panel, Fast 96-well	A15876
TaqMan Human Stem Cell Pluripotency Array	4385344
TRA-1-60 Antibody	411000
TRA-1-81 Antibody	411100

## References

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- Tsankov AM, Akopian V, Pop R et al. (2015) A qPCR Scorecard quantifies the differentiation potential of human pluripotent stem cells. *Nat Biotechnol* 33(11):1182–1192.

Find out more at [thermofisher.com/stemcells](http://thermofisher.com/stemcells)

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