

## Pluripotent stem cell guidebook

Key products and services for PSC research





Gibco™ media, supplements, cells, and cell culture reagents are designed to deliver reproducibility and performance for results you can count on every day. Regardless of whether you're performing cell culture in research labs or need a special or defined formulation, or are growing cell lines, primary, or stem cells, Gibco™ products are the reliable solution.



### Contents

#### **Stem cell introduction**

2 Somatic and progenitor cells product selection guide

#### Reprogramming

- 4 Reprogramming product selection guide
- 5 CytoTune-iPS 2.0 Sendai Reprogramming Kit

#### **Transfection**

- 8 Transfection product selection guide
- 9 Lipofectamine 3000 Transfection Reagent
- 10 Lipofectamine MessengerMAX Transfection Reagent

#### **Genome editing**

- 12 Genome editing product selection guide
- 13 CRISPR

#### **PSC** culture

- 16 PSC culture medium product selection guide
- 17 Essential 8 Medium
- 18 Essential 8 Flex Media System
- 19 PSC culture matrices product selection guide
- 20 PSC cryopreservation product selection guide
- 21 Characterization tools for PSC culture
- 22 TaqMan hPSC Scorecard Panel

#### **Differentiation**

- 24 Differentiation product selection guide
- 25 PSC Neural Induction Medium
- 27 PSC Dopaminergic Neuron Differentiation Kit
- 29 PSC Cardiomyocyte Differentiation Kit
- 31 PSC Definitive Endoderm Induction Kit
- 33 Differentiation growth factors

#### Characterization

- 35 Characterization and analysis tools
- 37 Select instruments for stem cell characterization and analysis

#### Services and support

- 40 CellModel Services
- 41 Pluripotent stem cell education
- 42 Gibco Pluripotent Stem Cell Workshops
- 43 Product ordering information

# 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. These areas of research require technologies that are not only efficient and reproducible, but also preserve the genetic integrity of the cells. 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. The unique aspect of these products is that they can be valuable in applications ranging from basic research to cell therapy research.

Advances in stem cell research have enabled scientists to start with somatic or progenitor cells, reprogram them into induced pluripotent stem cells (iPSCs), and then subsequently differentiate the PSCs into a variety of cell types. We offer a wide range of products and services that allow you to simplify your workflow and provide 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** 

### SUPPORT RESOURCES:

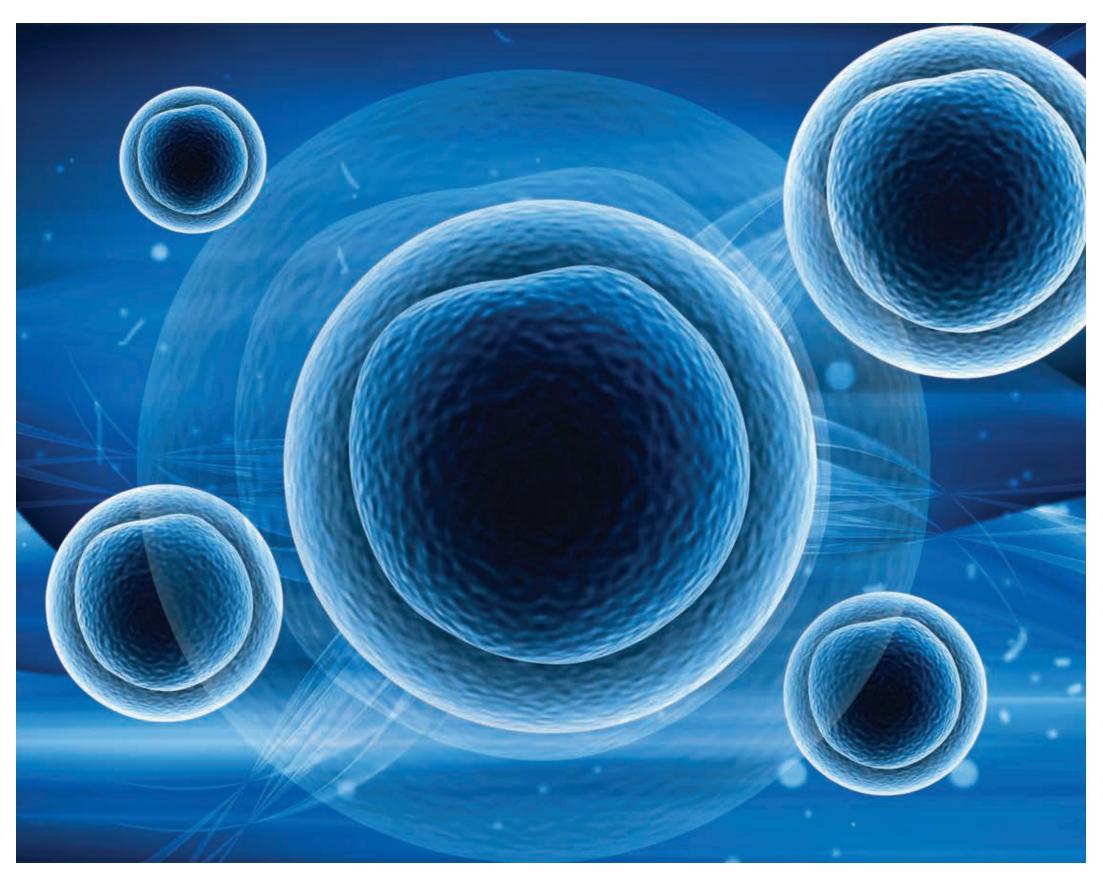
- To request the MSC Sourcebook, a product reference guide supporting your MSC/ADSC workflow, go to thermofisher.com/mscbook
- View stem cell protocols for expanding somatic cells at thermofisher.com/ stemcellprotocols

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

Cell type	ADSC	MSC	CD34+ and PBMC	РВМС	T cell	NSC	Human fibroblast	
Human adult stem and primary cells	Gibco StemPro Human Adipose- Derived Stem Cells Cat. No. R7788115	Gibco StemPro BM Mesenchymal Stem Cells Cat. No. A15652	Available in media kit	NA	NA	Gibco StemPro Neural Stem Cells Cat. No. A15654	Gibco Human Dermal Fibroblasts, Adult Cat. No. C-013-5C	
Recommended culture media	Gibco StemPro Human Adipose- Derived Stem Cell Kit Cat. No. R7788110	Gibco StemPro MSC SFM XenoFree Cat. No. A10675-01	Gibco StemPro CD34+ Cell Kit Cat. No. A14059	Gibco StemPro-34 SFM Cat. No. 10639-011	Gibco CTS OpTmizer T Cell Expansion SFM Cat. No. A10485-01*	Gibco StemPro NSC SFM Cat. No. A1050901	Gibco Essential 8 Medium Cat. No. A1517001	
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	Defined media for fibroblast expansion and reprogramming	
Antibodies	Find antibodies for all stem cell targets at <b>thermofisher.com/antibodies</b>							

Unless otherwise noted, all products are For Research Use Only. Not for use in diagnostic procedures.

<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.



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### Reprogramming

Reprogramming somatic cells to induced PSCs 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 preand post-reprogramming and detection of pluripotency in expanding 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.

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	CellModel Services
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	Reprogrammed cells and world-class support, delivered to you
Reprogramming efficiency	0.002-0.08%	0.04-0.3%	0.02-1.2%	0.02-1.2%
Genes utilized	Thomson/Yamanaka Factors	Yamanaka Factors + Lin28	Yamanaka Factors	Yamanaka Factors
Blood reprogramming	Yes (with Neon system only)	Yes (with Neon system only)	Yes	Yes
Delivery method	Neon electroporation	Lipofectamine 3000 Transfection Reagent-based	Transduction	Transfection or transduction
Cat. No.	A14703	A15960	A16517 (1 pack) A16518 (3 pack)	Please inquire: discoveryservices @thermofisher.com

 $<sup>^{\</sup>star}$  Commercialized in partnership with Cellular Dynamics International.

For Research Use Only. Not for use in diagnostic procedures.

 $<sup>^{\</sup>star\star}$  Designed by CiRA/Dr. Okita of CiRA/the Yamanaka Lab at CiRA/Kyoto University.

### CytoTune-iPS 2.0 Sendai Reprogramming Kit

### Highest 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 new temperature-sensitivity mutations to the polymerase-related genes, which help to clear the virus faster after reprogramming and cause less cytotoxicity to the cells.

This superior system enables:

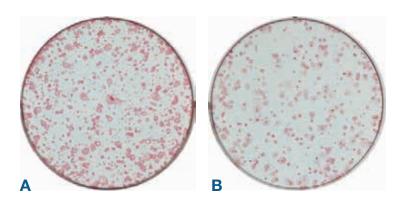
- The highest success rate for both fibroblast and blood reprogramming [1]
- Scalable cell line generation with minimal hands-on time
- Rapid clearance of RNA vectors

For more information on CytoTune reprogramming, visit thermofisher.com/cytotune

Prefer to focus on research goals besides reprogramming?

Let us do it for you with our Applied Biosystems<sup>™</sup> CellModel<sup>™</sup> Services—Stem Cell Reprogramming. Find out more at thermofisher.com/cellmodel





Fibroblast 0.49% efficiency

CD34<sup>+</sup> 0.46% efficiency

**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+ 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.

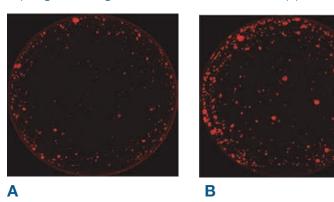


Figure 2. Improvement of feeder-free reprogramming efficiency using Gibco™ RevitaCell™ Supplement on day 7 transfer. Feeder-free reprogramming of human dermal neonatal fibroblasts (HDFn) (Cat. No. C-004-5C) was completed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at an MOI of 5:5:3. On day 7 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™

### Characterization tools for reprogramming

#### 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

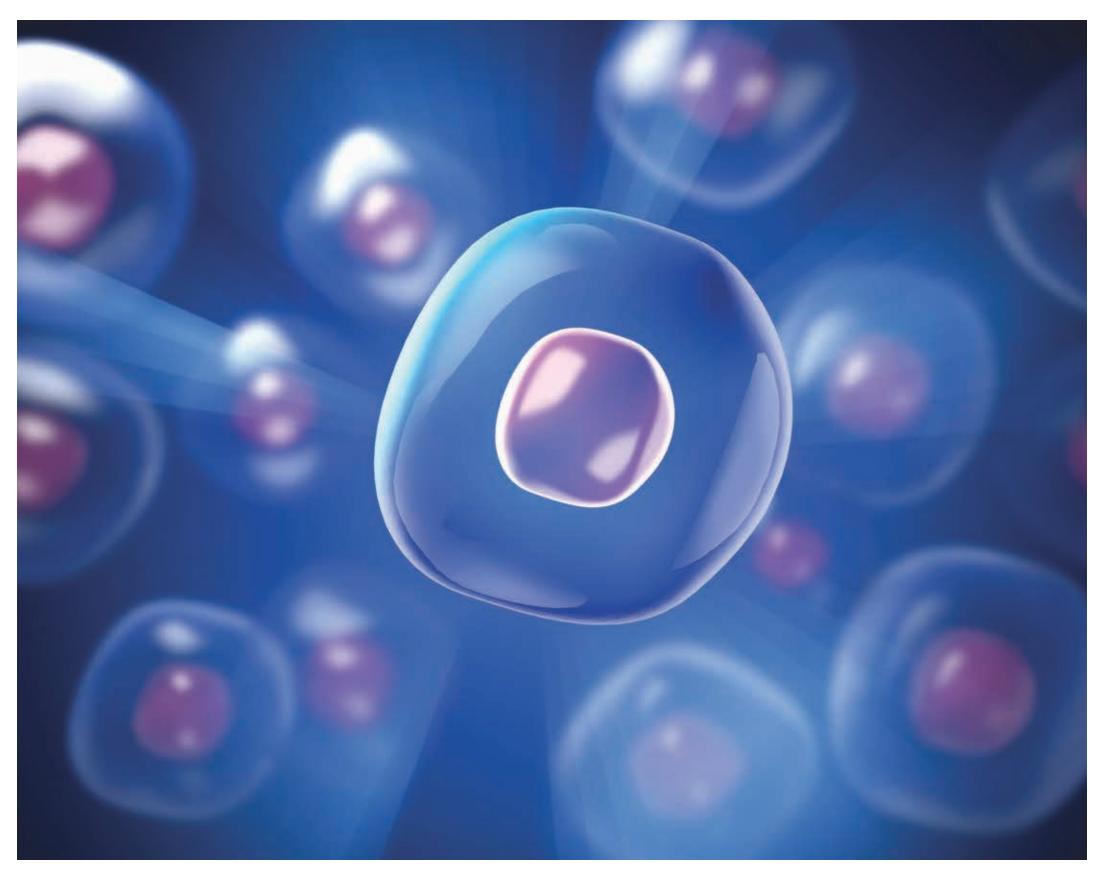
#### Live-cell immunostaining

Medium alone.

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

Gibco pluripotent stem cell guidebook



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### **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 3. Transfection 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
Superior transfection reagents								
Lipofectamine 3000 Transfection Reagent			闫					
Lipofectamine RNAiMAX Transfection Reagent			目					
Lipofectamine MessengerMAX Transfection Reagent		7						
Electroporation								
Neon Transfection System		7	E		= (=)			
CRISPR gene editing								
Lipofectamine 3000 Transfection Reagent								
Lipofectamine MessengerMAX Transfection Reagent		=)				Not yet tested		
Lipofectamine CRISPRMAX Cas9 Transfection Reagent				200		Not yet tested		

### Lipofectamine 3000 Transfection Reagent

### 10-fold higher transfection efficiency into difficult-to-transfect cells

Invitrogen™ Lipofectamine™ 3000 Transfection Reagent was developed to unleash the power of stem cells by providing a highly-efficient, cost-effective nucleic acid delivery alternative to electroporation (Figure 3). This advanced lipid nanoparticle technology 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:

- Superior efficiency—for the broadest spectrum of difficult-to-transfect cells (Figure 4)
- Low toxicity—gentle on cells for improved viability
- Versatility—single kit for DNA, RNA, and cotransfection

Learn more at thermofisher.com/3000

### Generate induced pluripotent stem cells (iPSCs)

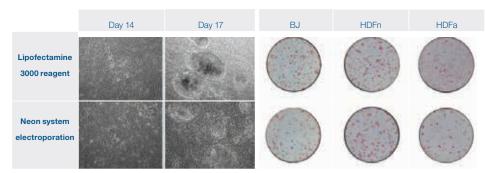
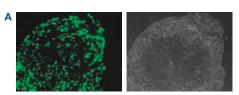
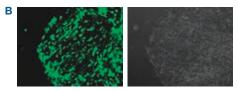


Figure 3. Reprogramming efficiency of Lipofectamine 3000 reagent compared to electroporation. BJ fibroblasts, as well as neonatal (HDFn) and adult (HDFa) human dermal fibroblasts, were reprogrammed to iPSCs by transfection of Invitrogen™ Epi5™ vectors using either Lipofectamine 3000 reagent or the Invitrogen™ Neon™ Transfection System. Colonies were visualized by brightfield microscopy and stained for alkaline phosphatase.

#### Transfect stem cells



DNA: 1.0 μg Lipofectamine 3000: 1.5 μL SSEA4\*/GFP\*: 42% GFP MFI: 247344



DNA: 1.3 µg Lipofectamine 3000: 1.5 µL SSEA4\*/GFP\*: 69% GFP MFI: 456741

#### Figure 4. Transfection of stem cells.

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

# Lipofectamine MessengerMAX Transfection Reagent

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

Introducing Invitrogen<sup>™</sup> Lipofectamine<sup>™</sup> MessengerMAX<sup>™</sup> mRNA Transfection Reagent with 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

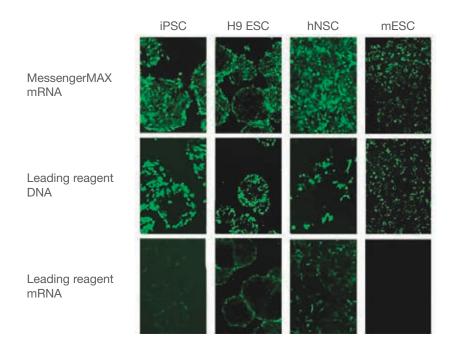
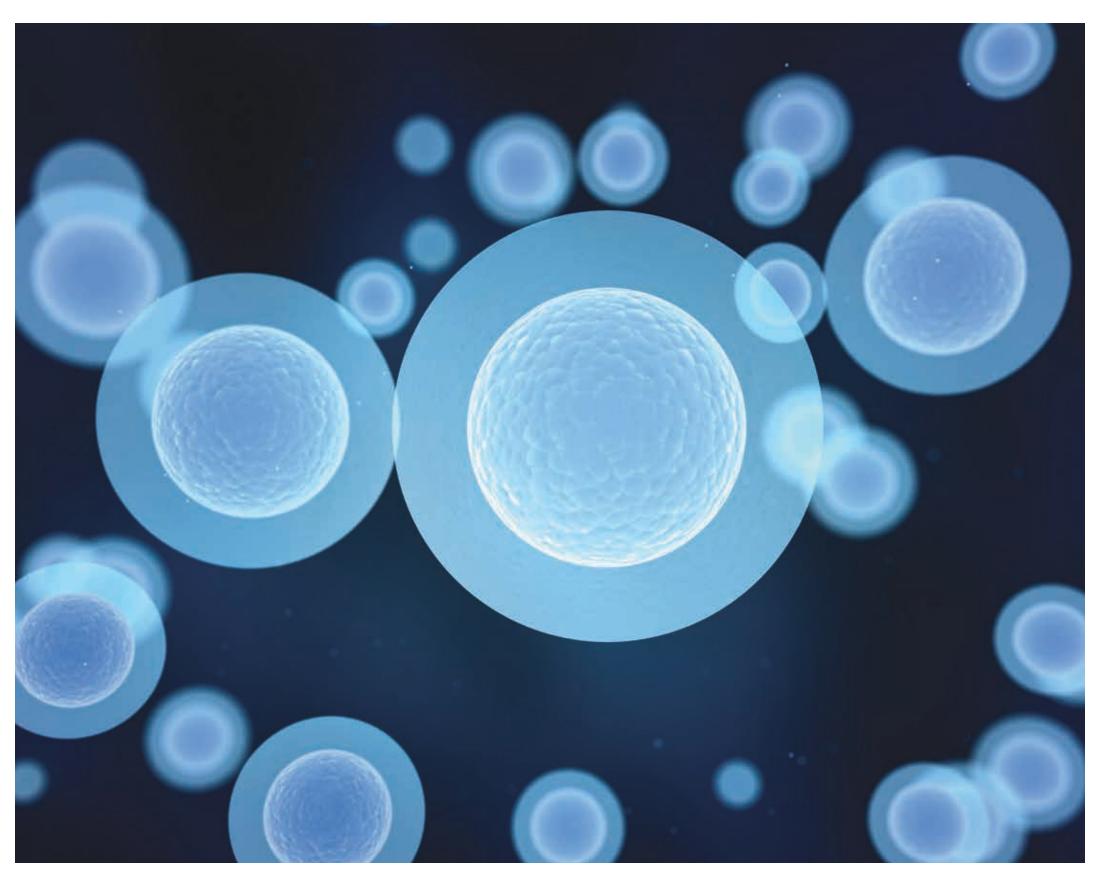


Figure 5. Lipofectamine MessengerMAX reagent outperforms leading DNA delivery reagent and leading mRNA delivery reagent in various stem cells (Gibco™ iPSCs, H9 ESCs, mESCs, and hNSCs). Lipofectamine MessengerMAX and the leading mRNA delivery reagent were used to deliver GFP mRNA (250 ng/well) in a 48-well format. The leading DNA delivery reagent was used to deliver GFP DNA (250 ng/well), and GFP was analyzed 24-hours posttransfection.



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### **Genome editing**

Genome editing—precise, site-specific DNA modification—can now be achieved through the use of technology derived from clustered regularly interspaced short palindromic repeats (CRISPRs) and transcription activator-like (TAL) effectors. CRISPR sequences target specific CRISPR RNA (crRNA) regions (or target-specific CRISPRs) and TAL effector DNA binding domains target nucleases to specific sites in the genome, creating double-strand breaks (DSBs) at desired locations (Table 4).

The natural repair mechanisms of the cell heal the break by either homology driven repair (HDR) or non-homologous end-joining (NHEJ). HDR is leverage for more precise edits since it utilizes a template allowing for introduction of foreign DNA carrying a user-defined sequence. DSB repair by NHEJ is likely to introduce errors, such as insertions or deletions (indels), leading to a nonfunctional gene.

### SUPPORT RESOURCES:

- Download the genome modulation and editing eBook at thermofisher.com/ geneengineeringebook
- Access the genome editing selection guide at thermofisher.com/ geneengineeringguide
- Find all the genome editing support you need at thermofisher.com/ genomeedit
- New to genome editing? Download our latest genome editing tips and tricks series at thermofisher.com/ genomeedittips

Table 4.
Gene editing product overview.

Product Name	GeneArt CRISPR all-in-one plasmid	GeneArt CRISPR mRNA	GeneArt Cas9 Protein	GeneArt CRISPR lentiviral libraries	GeneArt TAL effectors
Product benefits	All-in-one plasmid expressing Cas9 and gRNA     Contains reporter system for enriching transfected population	Multiplexing and screening capable     No cell-specific promoter constraint     No random integration concern	Multiplexing and screening capable     Superior cleavage efficiency     Fast turnover in cells limits nonspecific cutting     No cell-specific promoter constraint     No random integration concern	Infect dividing and nondividing mammalian cells     Provide long-term expression of CRISPR gRNA     Loss-of-function screening	The only provider of TALEN technology that includes the rights under foundational TAL IP  Precise Flexible; no sequence restriction or PAM requirement
Modification options	Gene knockout, Gene knock-in	Gene knockout, Gene knock-in	Gene knockout, Gene knock-in	Loss-of-function screening	Gene knockout, downregulation (knockdown), integration (knock-in), gene activation
Ease of design	Simple and fast design process	Simple and fast design process with gRNA synthesis kit, no need to clone	Simple and fast design process with gRNA synthesis kit, no need to clone	Ready-to-use lentiviral particles	Flexible; no design restrictions
Multiplexing	Not recommended	Capable	Capable	High-throughput screening	Rarely used
Design requirement	PAM site (NGG)	PAM site (NGG)	PAM site (NGG)	PAM site (NGG)	Active range of spacing needed for effector activity; no design constraints
Type of recognition	RNA-DNA	RNA-DNA	RNA-DNA	RNA-DNA	Protein-DNA

### CRISPR

### Revolutionizing the field of genome editing

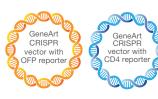
With their highly flexible yet specific targeting, CRISPR-Cas9 systems can be manipulated and redirected to become powerful tools for genome editing. CRISPR-Cas9 technology permits targeted gene editing in a variety of cells, and because the endonuclease cleavage specificity in CRISPR-Cas9 systems is guided by RNA sequences, editing can be directed to virtually any genomic locus by engineering the guide RNA sequence and delivering it along with the Cas9 endonuclease to your target cell. Our easy-to-use, optimized, and validated solutions span the entire stem cell engineering workflow, making genome editing accessible to anyone at any level.

We offer our state-of-the-art online Invitrogen™ CRISPR Search and Design Tool along with Invitrogen™ CRISPR-Cas9 editing products in four formats: an all-in-one expression vector, Cas9 mRNA, Cas9 protein, and CRISPR library services.

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

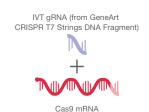
#### **Available CRISPR-Cas9 delivery formats**

The choice is yours: we offer a complete suite of tools and reagents



GeneArt CRISPR all-in-one plasmid





GeneArt CRISPR

mRNA



Lipofectamine MessengerMAX Transfection Reagent



GeneArt protein



Lipofectamine CRISPRMAX Transfection Reagent

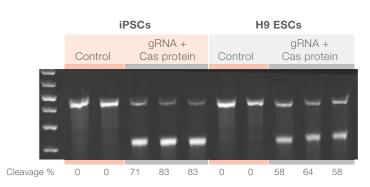
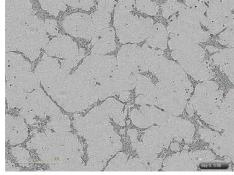


Figure 6. Genome editing of human stem cells with the Invitrogen™ GeneArt™ Cas9/gRNA RNP. Gibco™ human iPSCs and H9 ESCs were transfected in triplicate with GeneArt Cas9/gRNA ribonucleoprotein (RNP) complex, and target sites were analyzed for cleavage using the Invitrogen™ GeneArt™ Genomic Cleavage Detection (GCD) Kit 48–72 hours posttransfection. (See "Detection and analysis reagents".)

#### At the time of transfection



Posttransfection 48 hrs

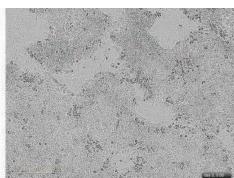


Figure 7. Invitrogen™ Lipofectamine™ CRISPRMAX™ Transfection Reagent achieves up to 55% cleavage efficiency, delivering 500 ng of Invitrogen™ GeneArt™ Platinum™ Cas9 Nuclease into iPSCs. All cells were culture in a 24-well plate format with Gibco™ Opti-MEM™ Serum Free Medium (Cat. No. 31985062) and captured via GCD assay.

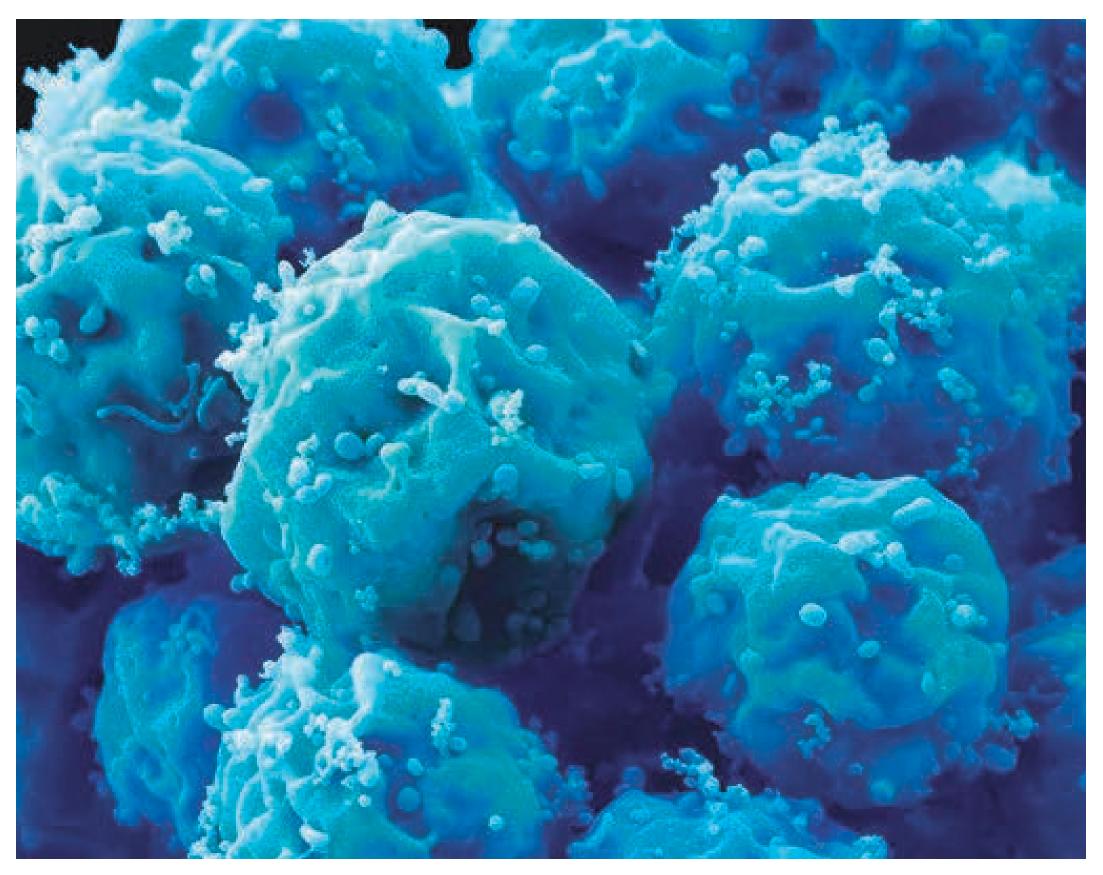
For a complete set of data that illustrate cutting efficiencies by cell line and delivery method, download our latest publication, "Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection", at **thermofisher.com/crisprprotein** 

### Characterization tools for gene editing

**GeneArt Genomic Cleavage Detection Kit**Direct detection of locus-specific genomic DNA modification.

Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Genomic Cleavage Selection Kit Quick screening of the cleavage activity and enrichment of nuclease-modified cells.

Find out more about these kits and other Invitrogen™ GeneArt™ product offerings at **thermofisher.com/geneart** 



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### 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 Gibco™ KnockOut™ Serum Replacement (KSR) to Gibco Essential 8 Medium and cell therapy research media, Gibco products deliver culture with confidence.

Visit **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 howto videos at thermofisher.com/ essential8howto

**Table 5.**Media systems for PSC culture.

Application	Feeder culture; reprogramming on feeders; differentiation of PSCs	Feeder-free culture	Feeder-free, xeno-free culture and expansion; feeder-free reprogramming and differentiation of PSCs	Feeder-based and feeder-free, xeno-free culture for cell therapy research
Medium	KnockOut Serum Replacement	Gibco StemPro hESC SFM	Essential 8 media systems	CTS KnockOut SR XenoFree Medium
Cat. No.	10828010* 10828028*	A1000701	thermofisher.com/ essential8media	12618-012* 12618-013*
Recommended substrate	Gibco Mouse (ICR) Inactivated Embryonic Fibroblasts	Gibco Geltrex hESC-qualified Ready-To-Use Reduced Growth Factor Basement Membrane Matrix	Gibco Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Gibco CTS CELLstart Substrate
Cat. No.	A24903	A1569601	A14700	A10142-01**
Recommended passaging technique	Gibco Collagenase IV or Gibco StemPro EZPassage Disposable Stem Cell Passaging Tool	Collagenase IV or StemPro EZPassage Disposable Stem Cell Passaging Tool	Gibco Versene Solution	Gibco CTS TrypLE Select Enzyme or EZPassage Disposable Stem Cell Passaging Tool
Cat. No.	17104-019 or 23181-010	17104-019 or 23181-010	15040-066	A12859-01 or 23181-010**

Unless otherwise indicated, all products are For Research Use Only. Not for use in diagnostic procedures.

<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.

### **Essential 8 Medium**

### Most defined and consistent stem cell culture conditions

Gibco 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, and can be used to scale up production of iPSCs. 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.

The Gibco™ Essential 8™ media system provides multiple xeno-free culture reagents for PSCs. This system offers the most defined conditions and enables more consistent results.

Learn more about the variations of Essential 8 Medium at thermofisher.com/essential8media

#### Where would you like to gain control?

Choose the Essential 8 media system that's right for you.



PSC expansion and maintenance **Essential 8 Medium** The most defined and consistent feeder-free medium for iPSCs



Essential 8 Medium + RevitaCell Supplement Control the level of stress on your cells



Flexible feeding schedule Essential 8 Flex Medium Manage your PSC culture schedule and eliminate daily feeding



Feeder-based transition
Gibco Essential 8
Adaptation Kit
Promote PSC survival
during the transition
from feeder-based to
feeder-free culture

### Essential 8 Flex Media System

### Manage your PSC culture schedule and eliminate daily feeding

Gibco™ Essential 8 Flex Medium is formulated to extend the activity of key heat-sensitive components found in PSC medium, including FGF2, to enable a weekend-free culture feeding schedule.

- Flexible feeding schedule—maintain pluripotency over a full 2-day period without feeding cells
- Easy to transition—same set-up, applications, and protocol as the original Essential 8 Medium formulation, just without the daily feeding
- Proven—based on the original Essential 8 Medium formulation, maintains pluripotency and normal karyotypes in long-term culture

Weekend-free feeding is possible—compare your PSC feeding schedule to Essential 8 Flex Medium.



The old way: Feed your cells every day, 7 days a week



#### The Flex way: Eliminate daily feeding



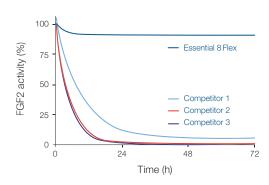


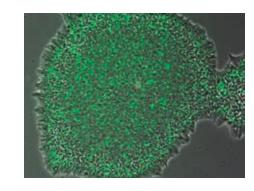
Figure 8. FGF2 activity over time in PSC culture medium. Unlike other feeder-free PSC culture media, Essential 8 Flex Medium has been optimized to extend the activity of unstable components such as FGF2. Extended FGF2 activity allows for routine culture without daily feeding.

### PSC culture matrices

We provide extracellular matrices (ECMs) that are designed to minimize adaptation time, maximize cell performance.

Whether you are culturing your cells on feeder layers or require feeder-free, xeno-free conditions for more demanding applications, Gibco products provide you with all the choices to culture your PSCs in the right environment.

Visit **thermofisher.com/pscmatrices** to find the right matrix for your research.



### **Table 6.** Extracellular matrices overview.

	Most commonly used basement membrane matrix for ESC and iPSC cultures	Simplified solution for the most commonly used basement membrane	Optimized for use with Essential 8 Medium	Superior recovery during challenging transitions
Product name	Gibco Geltrex LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix Cat. No. A1413301	Geltrex hESC-qualified Ready- to-Use Reduced Growth Factor Basement Membrane Matrix Cat. No. A1569601	Vitronectin (VTN-N) Recombinant Human Protein, Truncated Cat. No. A14700	rhLaminin-521 Cat. No. A29249
Application	Feeder-free culture	Feeder-free culture	Feeder-free and xeno-free culture	Feeder-free and xeno-free culture
Recommended medium pairing	StemPro hESC SFM or Essential 8 Medium	StemPro hESC SFM or Essential 8 Medium	Essential 8 Medium	Essential 8 Medium
Source origin	Cultured mouse EHS tumor cells; undefined	Cultured mouse EHS tumor cells; undefined	Human (recombinant); defined	Human (recombinant); defined

### PSC cryopreservation

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

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

### NEED MORE EFFICIENT RECOVERY?

RevitaCell Supplement has been optimized for use with PSCs as a post-thaw recovery solution to improve cell viability.

Find out more at

thermofisher.com/revitacell

### **Table 7.**Cryopreservation solutions overview.

	Gibco PSC Cryopreservation Kit	Gibco Synth-a-Freeze Cryopreservation Medium	Gibco Recovery Cell Culture Freezing Medium
	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
Tested cell types	iPSCs, ESCs, PBMCs, iPSC-derived cardiomyocytes	Human keratinocytes, MSCs, NSCs, other primary cell types	CHO-S, CHO-K1, HEK 293, Jurkat, NIH 3T3
Chemical composition	Xeno-free cryomedium; animal origin-free, chemically defined recovery supplement	Animal origin-free	Contains FBS
Ready to use	Yes	Yes	Yes
Recovery component included	Yes	No	No
CTS product available	NA	Gibco CTS Synth-a-Freeze Cryopreservation Medium Cat. No. A13713-01	NA
Cat. No.	A2644601	A12542-01	12648-010

### 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 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** 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 for characterization of undifferentiated stem cells	Evaluates pluripotency and confirms trilineage differentiation potential
Product Name	Alkaline Phosphatase Live Stain	Antibody staining	Applied Biosystems TaqMan Human Stem Cell Pluripotency Array	TaqMan hPSC Scorecard Panel
Cat. No.	A14353	thermofisher.com/ pscimmunokits	4385344	thermofisher.com/ scorecard
How specific are the results?	Low (stains stem and progenitor cells)	Medium (stains human ES and iPS cells)	Medium (profiles expression of human PSC and tissue makers)	High (profiles expression of human PSC and early germ layer markers)
Will the cells remain viable?	Yes	No	No	No
How long before I see results?	Stain PSCs typically in 20 minutes or less	Stain PSCs typically in 90–120 minutes	4–6 hours	6–8 hours
Are data analysis tools included?	No	No	No	Yes, free cloud-based software
Is a reference standard included?	No	No	No	Yes
Are EVOS FLoid cell imager protocols available?	Yes	Yes	NA	NA

### 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** 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.

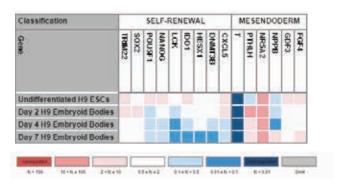


Figure 10. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.

### Invitrogen™ Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit

This kit enables optimal image-based analysis of four key markers of human hPSCs: OCT4, SOX2, SSEA4, TRA-1-60. This high performance immunocytochemistry kit includes a complete set of primary and secondary antibodies, a nuclear DNA strain, and premade buffers for an optimized staining experiment.

#### Invitrogen™ 3-Germ Layer Immunocytochemistry Kit

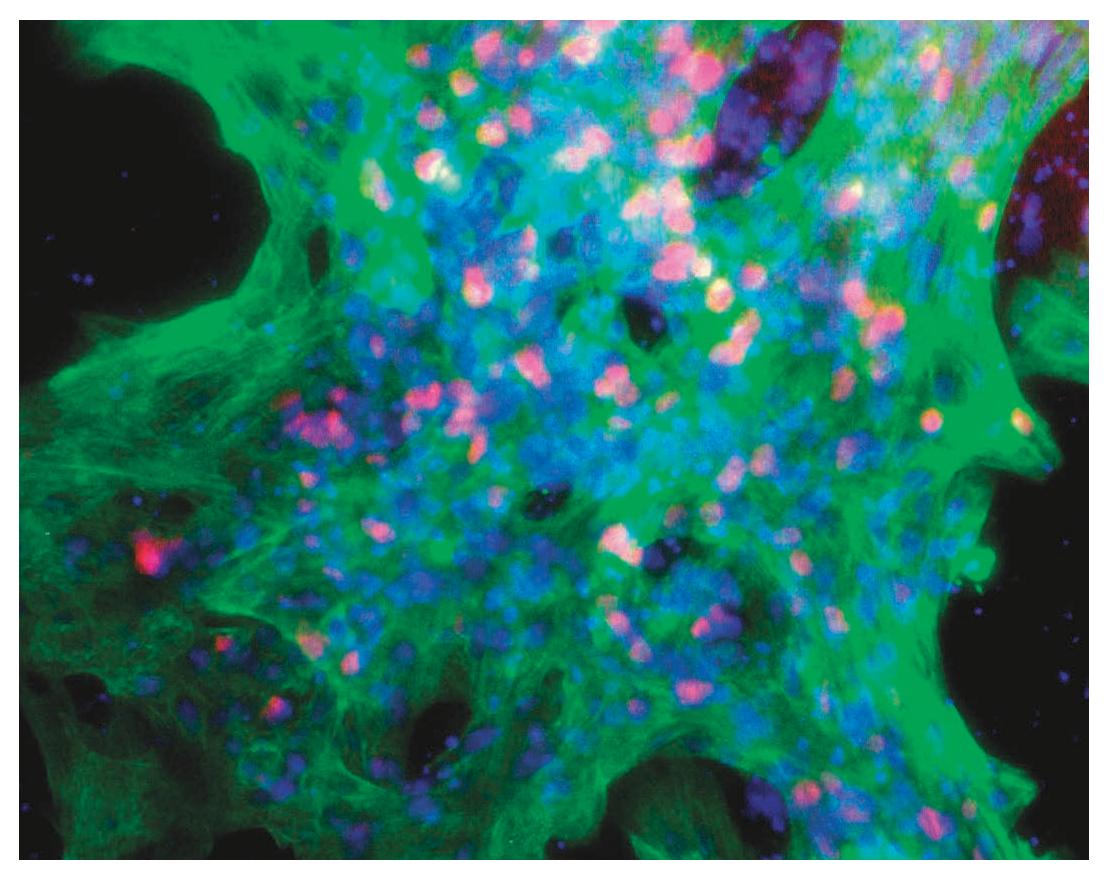
This kit enables optimal image-based analysis of spontaneously differentiated embryoid bodies from hPSCs. It detects widely accepted markers characteristic of the three embryonic germ layers: beta-III tubulin for ectoderm, smooth muscle actin for mesoderm, and alpha-fetoprotein for endoderm.

Learn more about these and additional staining kits at

thermofisher.com/pscimmunokits

#### With these kits, you can:

- Confirm expression of key markers with highly specific antibodies
- Get more information per sample by measuring more than one marker at a time
- Generate beautiful multiplexed images with minimal effort
- Save time and avoid cell loss by minimizing unnecessary wash steps in your staining protocols
- Process samples with confidence using a complete and optimized set of ICC reagents



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### **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.

### SUPPORT RESOURCES:

- View differentiation protocols at thermofisher.com/ stemcellprotocols
- View complete differentiation portfolio at thermofisher.com/differentiation

Table 9.
Media systems and reagents for differentiation.

	Ectoderm	Ectoderm	Mesoderm	Endoderm
Application	NSC differentiation	Dopaminergic neuron differentation	Cardiomyocyte differentiation	Definitive endoderm differentiation
Media system	Gibco PSC Neural Induction Medium Cat. No. A1647801	Gibco PSC Dopaminergic Neuron Differentiation Kit Cat. No. A30416SA	Gibco PSC Cardiomyocyte Differentiation Kit Cat. No. A2921201	Gibco PSC Definitive Endoderm Induction Kit Cat. No. A27654-SA
Substrate	Geltrex LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix Cat. No. A1413301	Vitronectin (VTN-N) Recombinant Human Protein, Truncated Cat. No. A14700 Laminin Mouse Protein, Natural Cat. No. 23017-015	Geltrex LDEV-Free hESC- qualified Reduced Growth Factor Basement Membrane Matrix Cat. No. A1413301	Vitronectin (VTN-N) Recombinant Human Protein, Truncated Cat. No. A14700
Assay duration	7 days	35 days	14 days	2 days
Cell type generated	Neural stem cells	Midbrain dopaminergic neurons	Cardiomyocytes	Definitive endoderm
Media format	50X supplement/500 mL basal, serum-free	Serum-free	Ready-to-use, xeno-free	Ready-to-use, xeno-free

### **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 11) 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 12). 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 13).

For more information, go to thermofisher.com/nscdiff



#### **Human NSC Immunocytochemistry Kit**

The Invitrogen™ Human Neural Stem Cell Immunocytochemistry Kit enables a convenient image-based analysis of four common markers of human neural stem cells: nestin, PAX6, SOX1, and SOX2. The kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and all of the premade buffers for an optimized staining experiment.

Learn more at thermofisher.com/pscimmunokits

#### With this kit, you can:

- Confirm expression of key markers with highly specific antibodies
- Get more information per sample by measuring more than one marker at a time
- Generate beautiful mutliplexed images with minimal effort
- Save time and avoid cell loss by eliminating unnecessary wash steps in your staining protocol

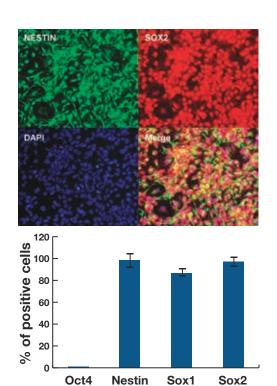


Figure 11. 80–90% neural induction efficiency.

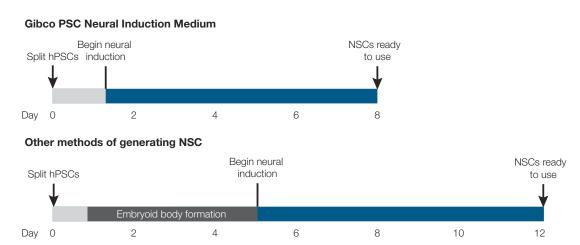


Figure 12. Unlike existing methodologies, Gibco PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation which adds time, labor, and variability.



Figure 13. Neural stem cells (NSCs) generated using Gibco PSC Neural Induction Medium have high expression of NSC markers and can be further differentiated into other neural cell types.

### 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

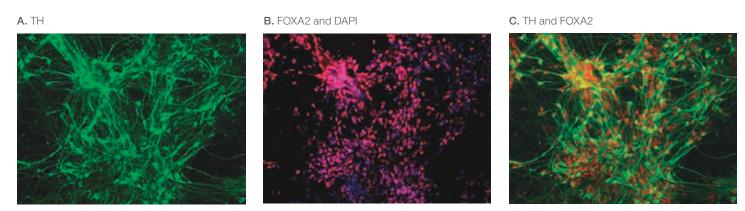


Figure 14. Representative images of mature DA neurons. The images were obtained from cells stained with reagents provided in the Invitrogen™ Human Dopaminergic Neuron Immunocytochemistry Kit (Cat. No. A29515) after 14 days of maturation of floor plate progenitor cells in Dopaminergic Neuron Maturation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

## Specification (Days 0–10)

## Expand/bank (Days 11–20)

## Maturation (Days 21–35)

- Start with PSCs cultured in Gibco Essential 8
   Medium
- Begin specification when cells are 20–40% confluent
- Change medium every 2 days

- Split cells and reseed in expansion medium
- Change medium every 2 days
- Cells can be frozen and banked at day 16 or continued to day 20 for maturation
- Dissociate cells and reseed in maturation media
- Change medium every 2 days
- Neurons can be visualized as early as day 30

Figure 15. 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.

### **Human Dopaminergic Neuron Immunocytochemistry Kit**

The Invitrogen Human Dopaminergic Neuron Immunocytochemistry Kit enables optimal image-based analysis of three key markers of neural differentiation: OTX2, FoxA2, and tyrosine hydroxylase (TH). The high-performance kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and premade buffers for an optimized staining experiment.

Find out more at thermofisher.com/pscimmunokits

#### With this kit, you can:

- Confirm expression of key cellular markers with highly specific antibodies
- Get more information per sample by measuring more than one marker at a time
- Generate beautiful multiplexed images with minimal effort
- Save time and avoid cell loss by minimizing unnecessary wash steps in your staining protocol

### 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/cardiacdiff



### Human Cardiomyocyte Immunocytochemistry Kit

The Invitrogen™ Human Cardiomyocyte Immunocytochemistry Kit enables optimal image-based analysis of 2 key cardiomyocyte markers: NKX2.5 and TNNT2/cTnT. The kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and all the premade buffers for an optimized staining experiment.

Learn more at thermofisher.com/pscimmunokits

#### With this kit, you can:

- Confirm expression of key markers with highly specific antibodies
- Get more information per sample by measuring more than one marker at a time
- Generate beautiful mutliplexed images with minimal effort
- Save time and avoid cell loss by eliminating unnecessary wash steps in your staining protocol
- Process samples with confidence using a complete and optimized set of immunocytochemistry reagents

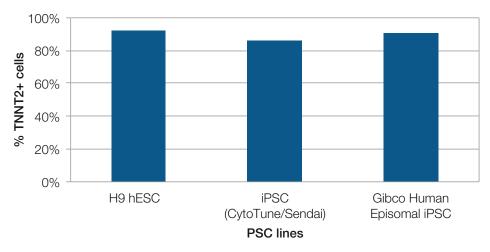


Figure 16. Efficiency across multiple PSC lines. Gibco™ TrypLE™-dissociated PSC lines were seeded at specific density onto Gibco™ Geltrex™-coated surface and cultured in Essential 8 Medium. After three days expansion, PSC lines at optimal confluency were induced using the PSC Cardiomyocyte Differentiation Kit according to protocol and cultured for two weeks. Cells were harvested and analyzed for TNNT2+ cells by flow cytometry. Results showed high cardiomyocyte differentiation efficiency among all lines when it reaches optimal confluency at time of induction.

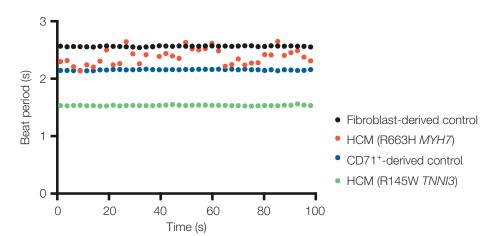


Figure 17. Electrophysiological assessment of hypertrophic cardiomyopathy patients' iPSC-derived cardiomyocytes generated using PSC Cardiomyocyte Differentation Kit on Maestro™ Multielectrode Array (MEA) platform (Axion Biosystems). The arrhythmic beating of the cardiomyocytes with mutation is evident when comparing its 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<sup>™</sup> 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+/PDGFR $\alpha$ - definitive endoderm cells with only 2 components in just 2 days (Figure 19).

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 20) and are capable of differentiating to downstream lineages.



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

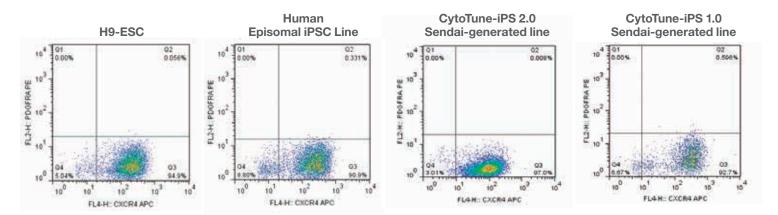
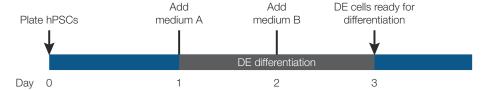


Figure 18. The PSC Definitive Endoderm Induction Kit produces DE populations with high efficiency (≥90%) across hESC and iPSC cell lines, including cell lines reprogrammed using episomal vectors or CytoTune kits. Representative dot plots show CXCR4+/PDGFRα− cell populations derived from various cell lines. For each experiment, unstained cells were used to set quadrant gates.

#### **PSC Definitive Endoderm Induction Kit**



#### Other commercial method of DE induction

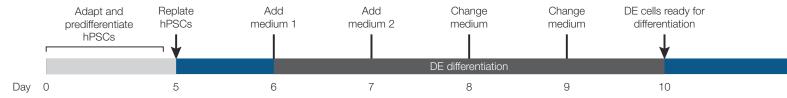


Figure 19. Compared to other differentiation protocols, the PSC Definitive Endoderm Induction Kit produces cells in up to 50% less time and requires no predifferentiation or mixing of media.

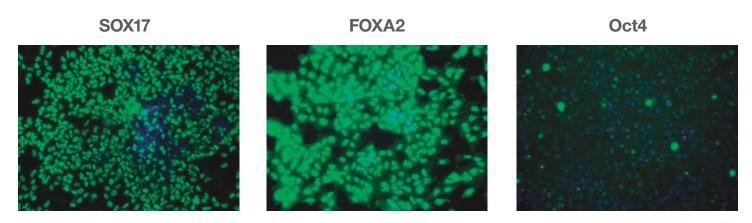
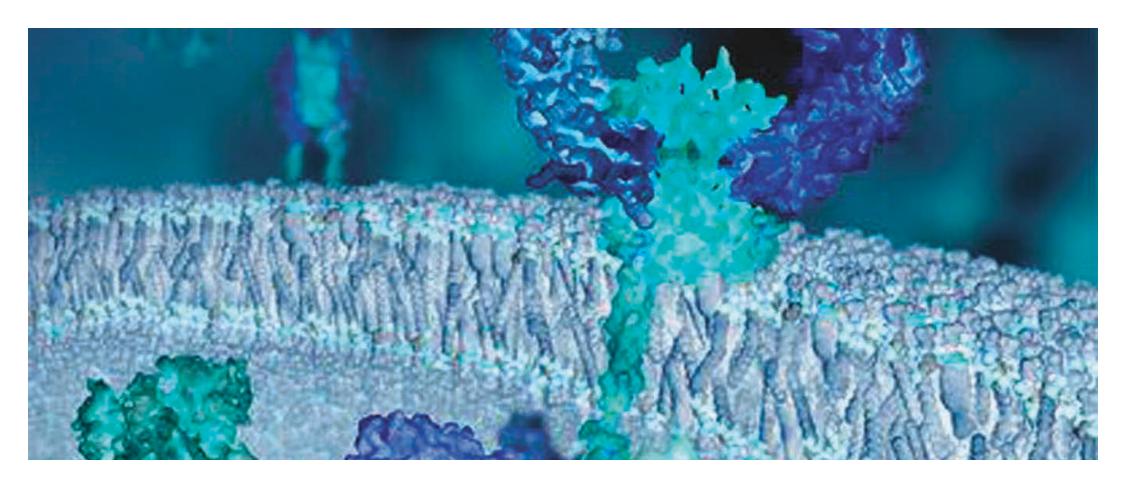


Figure 20. Immunocytochemistry of hESCs treated with the PSC Definitive Endoderm Induction Kit. At day 3, induced cells were immunostained for the endodermal transcription factors SOX17 and FOXA2, and the pluripotent marker Oct4. Nuclei were counterstained with DAPI (blue) to assess total cell numbers.



### **Differentiation: Gibco growth factors**

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

### 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 hematopoeisis, neural development, and inflammation.

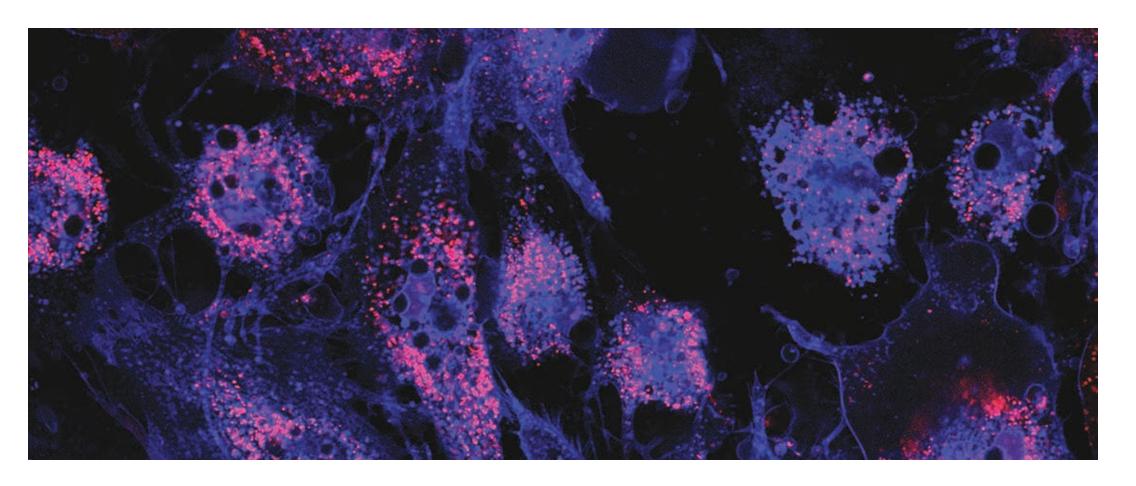
### **Tumor Necrosis Factor (TNF)**

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

### **Vascular Endothelial Cell Growth Factor (VEGF)**

VEGF exerts angiogenic, mitogenic, and vascular permeability—enhancing activities specific for endothelial cells. VEGF has also been shown to be chemotactic for monocytes and osteoblasts.

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



### **Characterization and analysis tools**

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

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

### **Labeling and detection tools**

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

- Invitrogen<sup>™</sup> Qdot<sup>™</sup> nanocrystals
- Invitrogen<sup>™</sup> Alexa Fluor<sup>™</sup> dyes
- Invitrogen<sup>™</sup> Alexa Fluor<sup>™</sup> secondary antibodies and streptavidin
- Primary antibodies
- Alkaline Phosphatase Live Stain
- Invitrogen<sup>™</sup> 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<sup>™</sup> TaqMan<sup>™</sup> protein analysis
- Invitrogen<sup>™</sup> multiplex assays
- Invitrogen<sup>™</sup> antibodies for western detection
- Invitrogen<sup>™</sup> ELISA kits
- Invitrogen<sup>™</sup> Bolt<sup>™</sup> protein separation and detection system
- Western blotting kits

### Sample preparation

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

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

### **Genomic analysis**

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

- Applied Biosystems<sup>™</sup> TaqMan<sup>™</sup> Gene Expression Assays
- Applied Biosystems<sup>™</sup> TaqMan<sup>™</sup> miRNA Assays
- Applied Biosystems<sup>™</sup> TaqMan<sup>™</sup> SNP Assays
- Applied Biosystems<sup>™</sup> TaqMan<sup>™</sup> CNV Assays
- Applied Biosystems<sup>™</sup> AuthentiFiler<sup>™</sup> PCR Amplification Kit
- Ion AmpliSeq<sup>™</sup> panels

### Pluripotent stem cell antibodies

No matter which application is used to characterize pluripotent stem cells—ChIP, ICC, IF, IHC, flow cytometry, western blot, or ELISA—there is an Invitrogen stem cell antibody for you. With over 40,000 antibodies covering many stem cell targets, you will be able to find the best antibody for your research.

Once there, use the antibody search tool and filter results by target, host species, monoclonal, or polyclonal antibody type, among other criteria.

Find antibodies for all stem cell targets by visiting

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** 



#### **Neon Transfection System**

The Invitrogen Neon Transfection System is a next-generation electroporation device for highly efficient transfection of primary cells, stem cells, and difficult-to-transfect cells. Unlike other electroporation instruments, the flexible and open system allows for high-quality transfections using optimized or user-defined protocols in three simple steps with as few as 2 x 10<sup>4</sup> cells per reaction. A novel chamber provides a dramatic increase in transfection efficiency and cell viability. Data- and cell-specific protocols are available at **thermofisher.com/neon** 



#### 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 just 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



### **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 unparalleled sample analysis speed up to 10X faster throughput than traditional cytometers with clogresistant engineering. Easily switch between tubes and plates in seconds and leverage the complete walkaway 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



### 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** 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** 



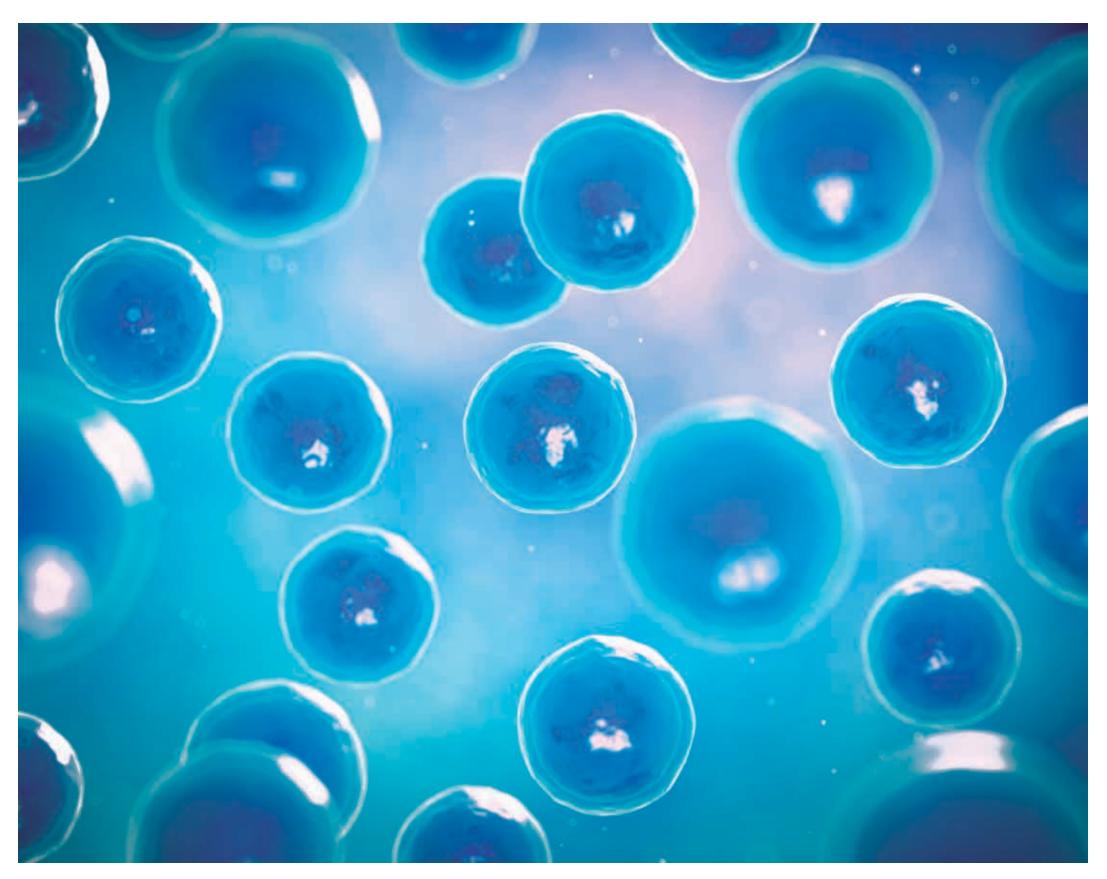
### Ion Personal Genome Machine (PGM) sequencer

Powered by Ion Torrent<sup>™</sup> semiconductor chip technology, the Ion Personal Genome Machine<sup>™</sup> (PGM<sup>™</sup>) sequencer delivers the fastest sequencing run times, at the most affordable price, of any next-generation sequencer. Go to **thermofisher.com/pgm** to learn more about the Ion PGM<sup>™</sup> System.



### 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** 



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### **CellModel Services**

Built on the stem cell innovations that we have introduced throughout the past decade, our Applied Biosystems™ CellModel™ Services enable stem cell scientists to reach their desired outcomes faster. We offer stem cell researchers choices at every stage of their research inclduing 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.



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

- 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

#### Available stem cell services

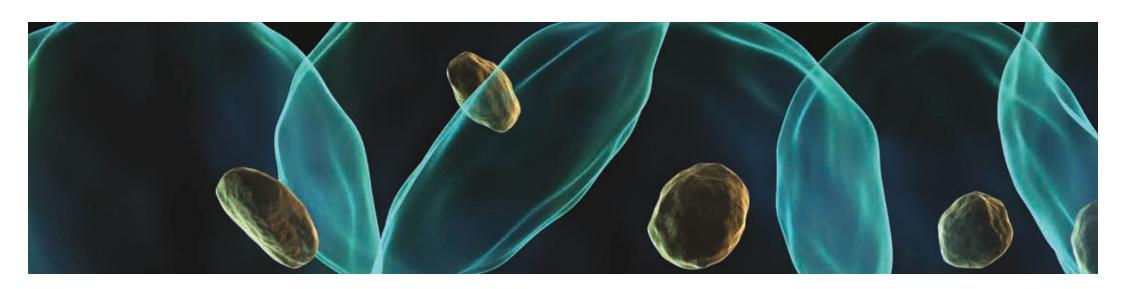
Choose the combination of tools and services that best fits your lab resources and expertise, including:

- Reprogramming—human fibroblasts or blood cells reprogrammed in 4 to 6 months with top clones expanded, cryopreserved, and characterized differentiation
- Differentiation—from pluripotent stem cells to your desired cell model using our suite of Gibco™ differentiation media
- Characterization—confirm pluripotency, gene expression, cell identity, and more
- Assay development—customized;
   validates stem cell-based assays for interrogation of disease-relevant biology
- Screening—compound screening for your stem cell-based discovery projects utilizing our complete high-throughput screening (HTS) capabilities

#### **Experienced service providers**

CellModel Services are delivered by scientists from our custom biology team who have years of experience helping researchers with their cellular engineering and custom assay development projects. Additionally, our discovery services project management team—responsible for the management of hundreds of cell line generation and assay development projects—is uniquely suited to manage your project milestones and proactively communicate updates from day one to project completion.

For more information, go to thermofisher.com/cellmodel



### 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:

- Free stem cell poster
- Gibco<sup>™</sup> Pluripotent Stem Cell Handbook
- Key industry events such as ISSCR and 24 Hours of Stem Cells
- Webinars from top researchers in the field

## Test your knowledge with pluripotent stem cell trainings and certifications including:

- Gibco<sup>™</sup> PSC Culture Virtual Lab and certification
- Gibco<sup>™</sup> PSC hands-on workshop and certification

# Apply your knowledge to your stem cell research with our solutions and resources including:

- Application notes
- Frequently asked questions
- How-to videos
- Protocols
- Publications
- Scientific posters
- Technical support

### Gibco 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** 

#### **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 feederfree 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.

### **Ordering information**

Product	Cat. No.
Somatic and progenitor cells	
CTS Immune Cell Serum Replacement*	A25961-01
Human Dermal Fibroblasts, Adult	C-013-5C
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
StemPro-34 SFM	10639-011
Reprogramming	
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	
ExpiFectamine 293 Transfection Kit	A14526
Invivofectamine 3.0 Reagent	IVF3001
Lipofectamine 2000 Transfection Reagent	11668-019
Lipofectamine 3000 Transfection Reagent	L3000-001
Lipofectamine MessengerMAX Transfection Reagent	LMRNA015
Lipofectamine RNAiMAX Transfection Reagent	13778-075
Genome editing	
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

Product	Cat. No.
Genome editing	
GeneArt CRISPR Nuclease Vector with OFP Reporter Kit (with competent cells)	A21178
GeneArt CRISPR T7 Strings DNA	Contact geneartsupport@ thermofisher.com
GeneArt CRISPR U6 Strings DNA	Contact geneartsupport@ thermofisher.com
GeneArt Genomic Cleavage Detection Kit	A24372
GeneArt Genomic Cleavage Selection Kit	A27663
GeneArt Platinum Cas9 Nuclease	B25640
GeneArt Precision gRNA Synthesis Kit	A29377
GeneArt TALs	Contact geneartsupport@ thermofisher.com
Culture	
Collagenase IV	17104-019
CTS TrypLE Select Enzyme <sup>†</sup>	A12859-01
Essential 6 Medium	A1516401
Essential 8 Flex Medium Kit	A2858501
Essential 8 Medium	A1517001
KnockOut Serum Replacement**	10828-028
RevitaCell Supplement	A26445-01
StemPro EZPassage Disposable Stem Cell Passaging Tool	23181-010
StemPro hESC SFM	A10007-01
Matrices	
CTS CELLstart Substrate <sup>†</sup>	A10142-01
Geltrex hESC-qualified Ready-To-Use Reduced Growth Factor Basement Membrane Matrix	A1569601

Product	Cat. No.
Matrices	
Geltrex LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A1413301
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Cryopreservation	
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
Differentiation	
Activin A Recombinant Human Protein	PHC9564
bFGF Recombinant Human Protein	13256029
EGF Recombinant Human Protein	PHG0311
GM-CSF Recombinant Human Protein	PHC2015
PSC Cardiomyocyte Differentiation Kit	A2921201
PSC Definitive Endoderm Induction Kit	A27654-SA
PSC Dopaminergic Neuron Differentiation Kit	A30416SA
PSC Neural Induction Medium	A1647801
TNF Recombinant Human Protein	PHC3015
VEGF Recombinant Human Protein	PHC9394
Characterization	
3-Germ Layer Immunocytochemistry Kit	A25538
AlexaFluor 488 CD44 Live Cell Imaging Kit	A25528
AlexaFluor 488 Tra-1-60 Live Cell Imaging Kit	A25618

Product	Cat. No.
Characterization	
AlexaFluor 555 Tra-1-60 Live Cell Imaging Kit	A24879
AlexaFluor 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
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

Unless otherwise indicated all products are For Research Use Only.

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.

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

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#### References

- 1. Schlaeger TM, Daheron L, Brickler TR et al. (2015) A comparison of non-integrating reprogramming methods. Nat Biotechnol 33(1):58-63.
- 2. Bock C, Kiskinis E, Verstappen G et al. (2011) Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144(3):439-452.
- 3. 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.

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