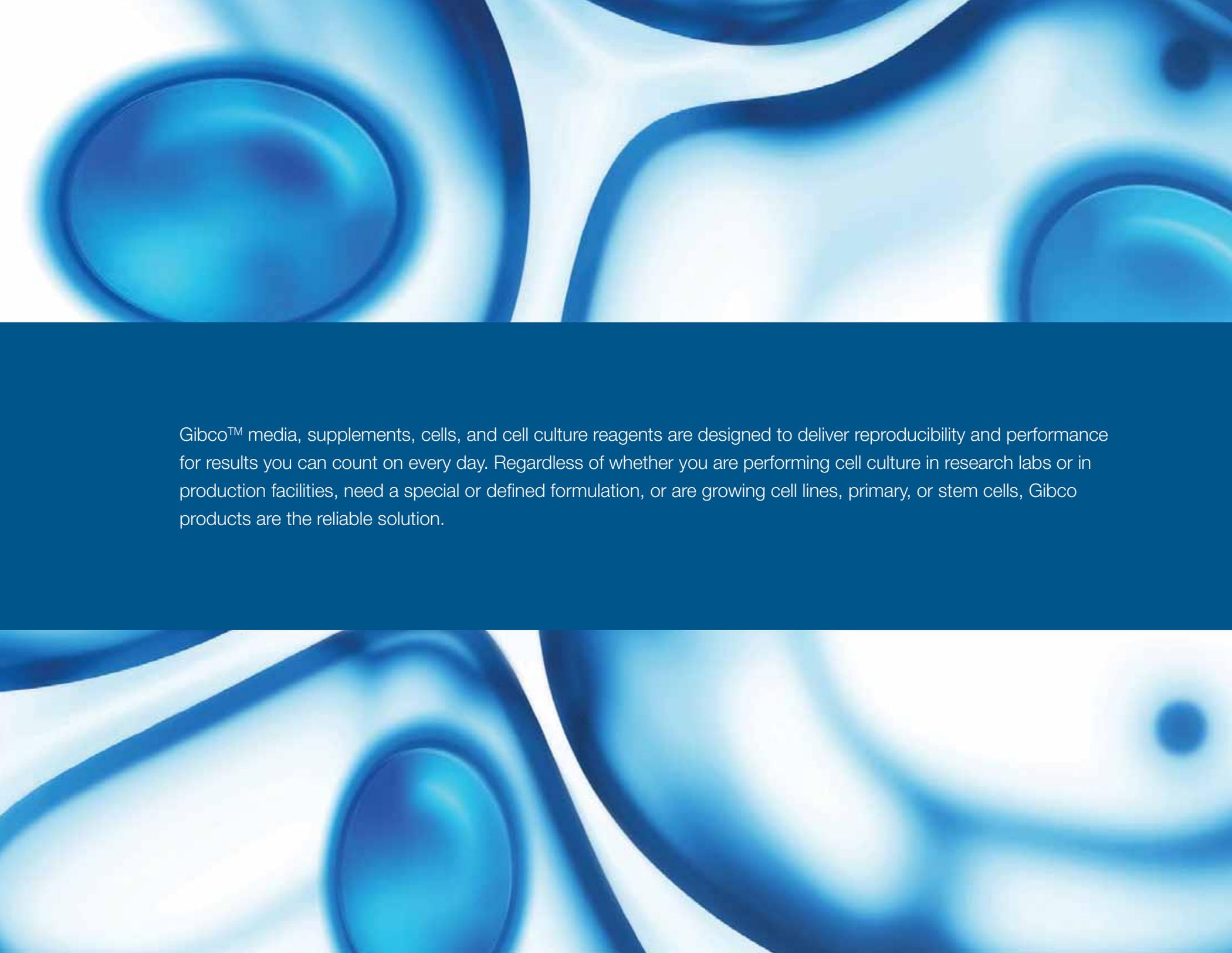


gibco

Pluripotent stem cell guidebook

Key products and services for PSC research

ThermoFisher
SCIENTIFIC

The image features a microscopic view of cells in culture, showing various cell shapes and structures. The cells are stained with a blue dye, highlighting their nuclei and other internal components. The background is a light blue, and the overall image has a soft, blurred quality. A dark blue horizontal band is overlaid on the image, containing white text.

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 are performing cell culture in research labs or in production facilities, need a special or defined formulation, or are growing cell lines, primary, or stem cells, Gibco products are the reliable solution.

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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. 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 differentiated 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 range of cells and expansion media backed by the Gibco™ brand, 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 available at thermofisher.com/stemcells

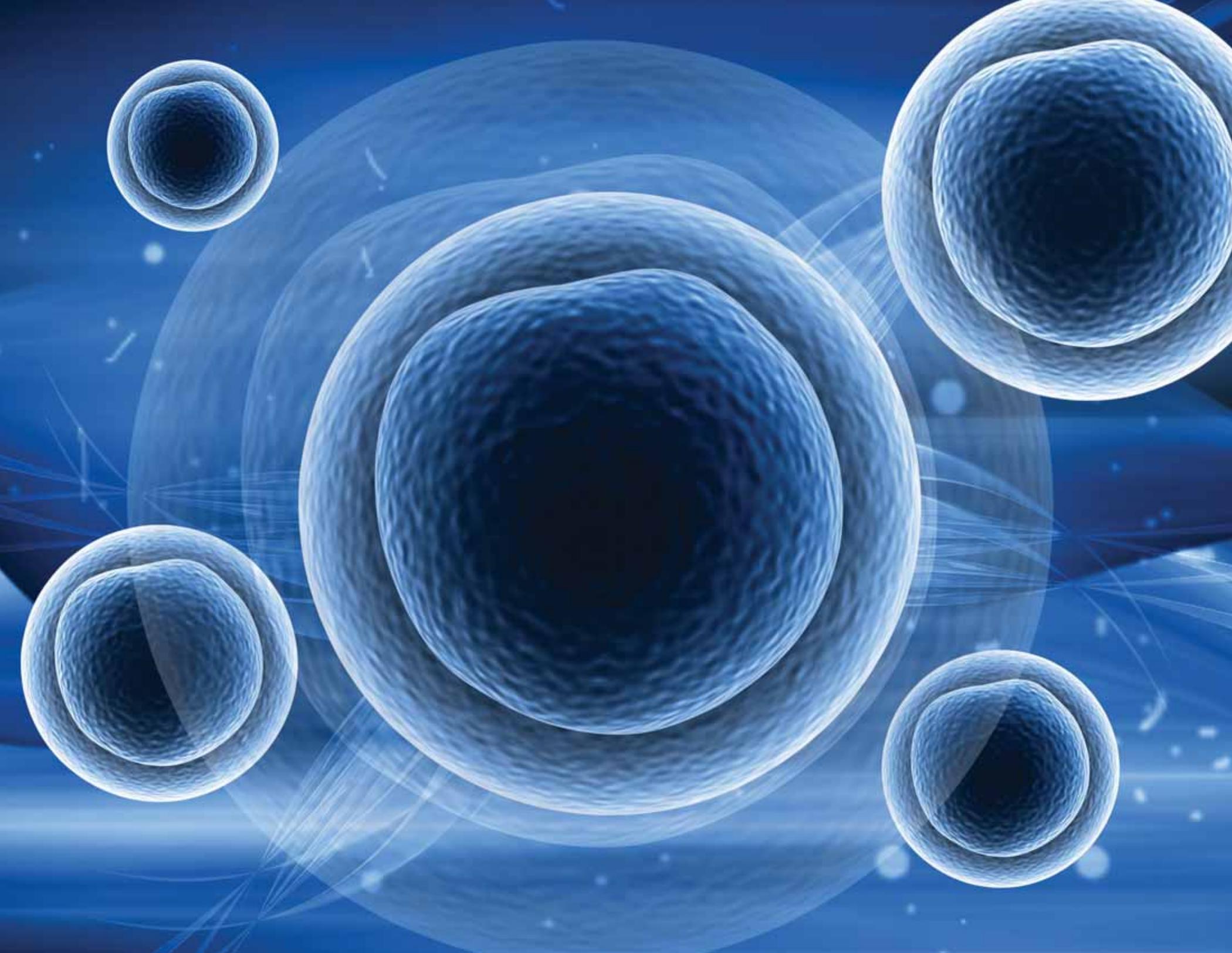
SUPPORT RESOURCES:

- Request the MSC Sourcebook—a product reference guide supporting your MSC/ADSC workflow. Request at thermofisher.com/mscbook
- View stem cell protocols for expanding somatic cells at thermofisher.com/stemcellprotocols

Table 1.
Somatic and progenitor
cell media overview.

Cell type	ADSC	MSC	CD34+ and PBMC	PBMC	T cell	NSC	Human fibroblast
Human adult stem and primary cells	Gibco™ StemPro™ Human Adipose-Derived Stem Cells Cat. No. R7788115	Gibco™ StemPro™ BM Mesenchymal Stem Cells Cat. No. A15652	Available in media kit	N/A	N/A	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 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

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



Reprogramming

Reprogramming somatic cells to induced pluripotent stem cells 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 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/cytotunerresources

Table 2.
Non-integrating reprogramming products and services overview.

Product name	Episomal iPSC Reprogramming Vectors*	Epi5™ Episomal iPSC Reprogramming Vectors**	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	None
Cat. No.	A14703	A15960	A16517 (1 pack) A16518 (3 pack)	Please inquire: discoveryservices@thermofisher.com

*Commercialized in partnership with Cellular Dynamics International.

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

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

CytoTune-iPS 2.0 Sendai Reprogramming Kit

Most efficient, non-integrating reprogramming technology available.

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 an increased 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:

- Increased reprogramming efficiency for even more colonies
- Lower cytotoxicity to allow for smaller starting cell populations
- Faster clearance to get your iPSC experiments sooner

For more information on CytoTune reprogramming, visit [thermofisher.com/cytotune](https://www.thermofisher.com/cytotune)



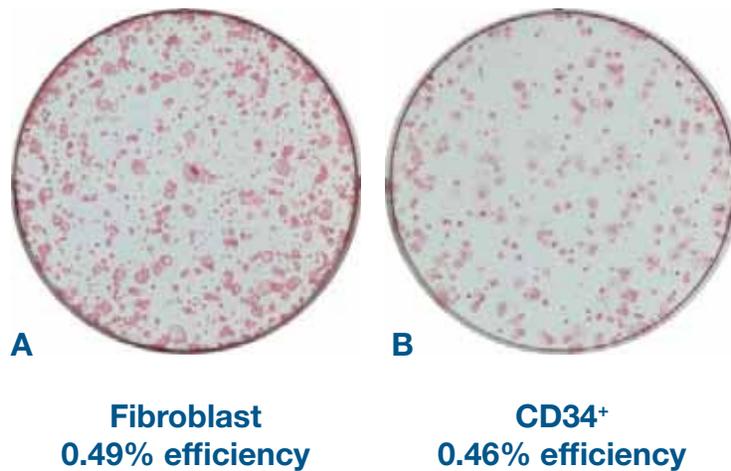


Figure 1. Reprogramming efficiency. Alkaline phosphatase staining of iPSCs generated from **(A)** human dermal neonatal fibroblasts (BJ strain) and **(B)** human umbilical cord blood–derived CD34+ cells, using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit at an MOI of 5:5:3, shown at 21 days post transduction.

Need even better reprogramming efficiency?

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

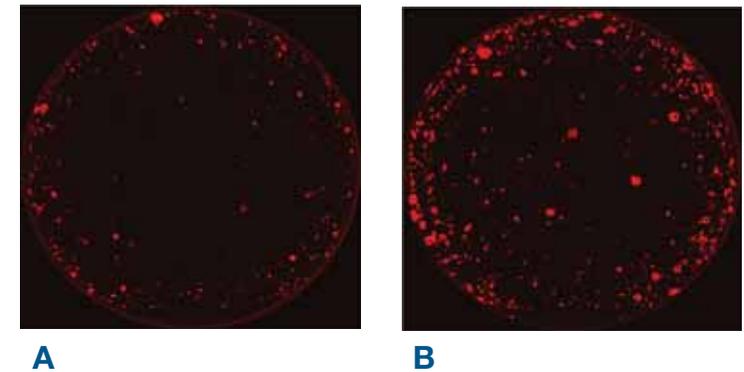


Figure 2. Improvement of feeder-free reprogramming efficiency using RevitaCell™ Supplement on Day 7 transfer. Feeder-free reprogramming of human dermal neonatal fibroblasts (HDFn) (Cat. No. C-004-5C) was completed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit at an MOI of 5:5:3. On Day 7 post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

Characterization tools for reprogramming

Alkaline Phosphatase Live Stain

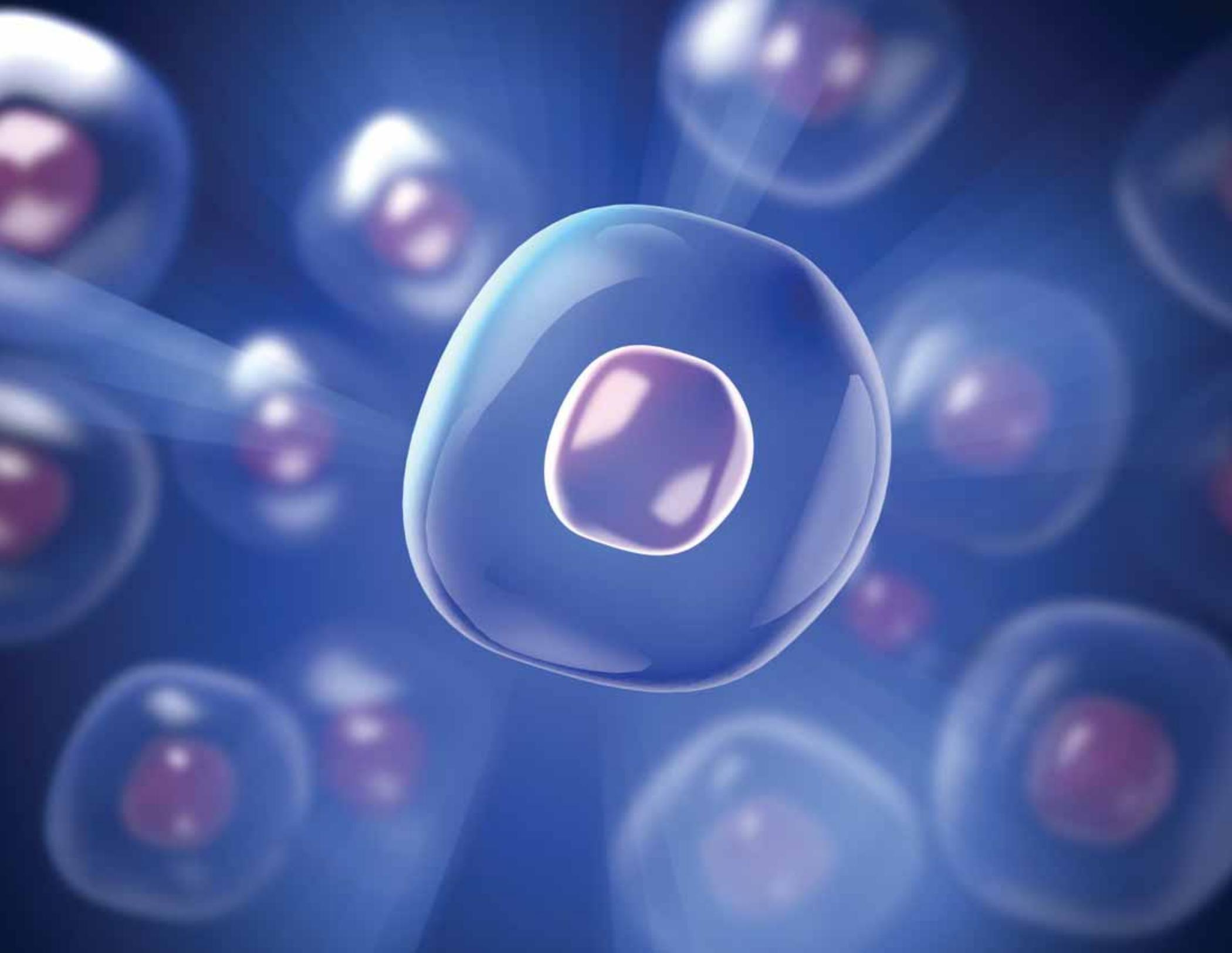
Invitrogen™ Molecular Probes™ Alkaline Phosphatase Live Stain is used for stem cell imaging that allows you to differentially stain PSCs. The dye is a cell-permeable fluorescent substrate for alkaline phosphatase (AP) that is nontoxic to cells, diffusing away over the course of 2 hours.

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)



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. 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://www.thermofisher.com/transfection)

SUPPORT RESOURCES:

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

Table 4.

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

Transfection product	DNA	mRNA	RNAi	Co-delivery*	Easy-to-transfect cells	Difficult-to-transfect cells	Primary cells	Stem cells	Suspension cells
Superior transfection reagents									
Invitrogen™ Lipofectamine™ 3000 Transfection Reagent					████████	████████	███████	███████	███████
Invitrogen™ Lipofectamine™ RNAiMAX Transfection Reagent					████████	████████	███████	███████	███████
Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent					████████	████████	███████	███████	███████
Broad-spectrum transfection reagent									
Invitrogen™ Lipofectamine™ 2000 Transfection Reagent					███████	███████	███████	███████	███████
Electroporation									
Invitrogen™ Neon™ Transfection System					████████	████████	███████	███████	███████
In vivo delivery									
Invitrogen™ Invivofectamine™ 3.0 Reagent									<i>In vivo delivery to liver following tail vein injection</i>

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 5)
- 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)

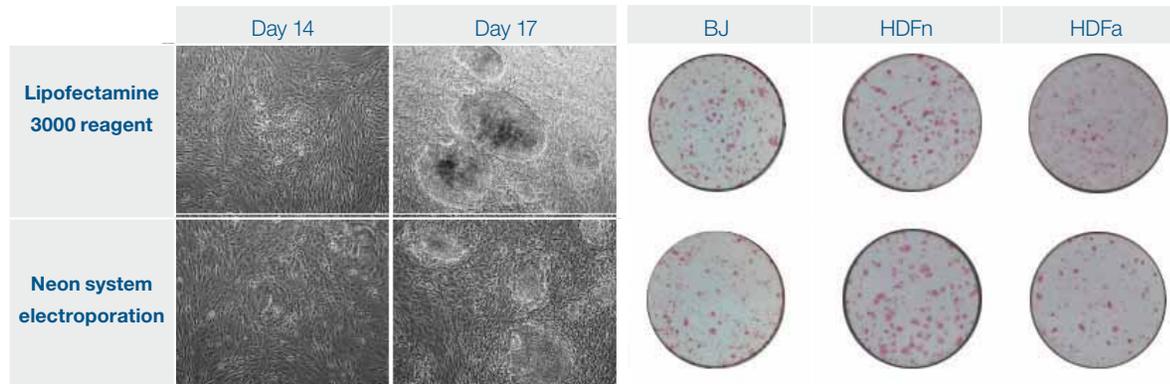


Figure 3. Reprogramming efficiency of Lipofectamine 3000 reagent compared to electroporation. BJ fibroblasts, as well as neonatal (HDFn) and adult (HDFa) human dermal fibroblasts, were reprogrammed to iPSCs by transfection of Epi5™ vectors using either Lipofectamine 3000 reagent or the Neon Transfection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

Transfect stem cells

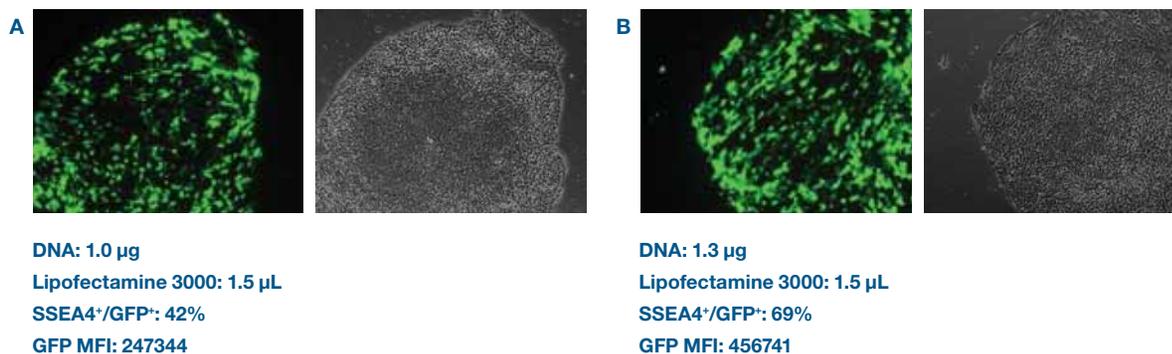
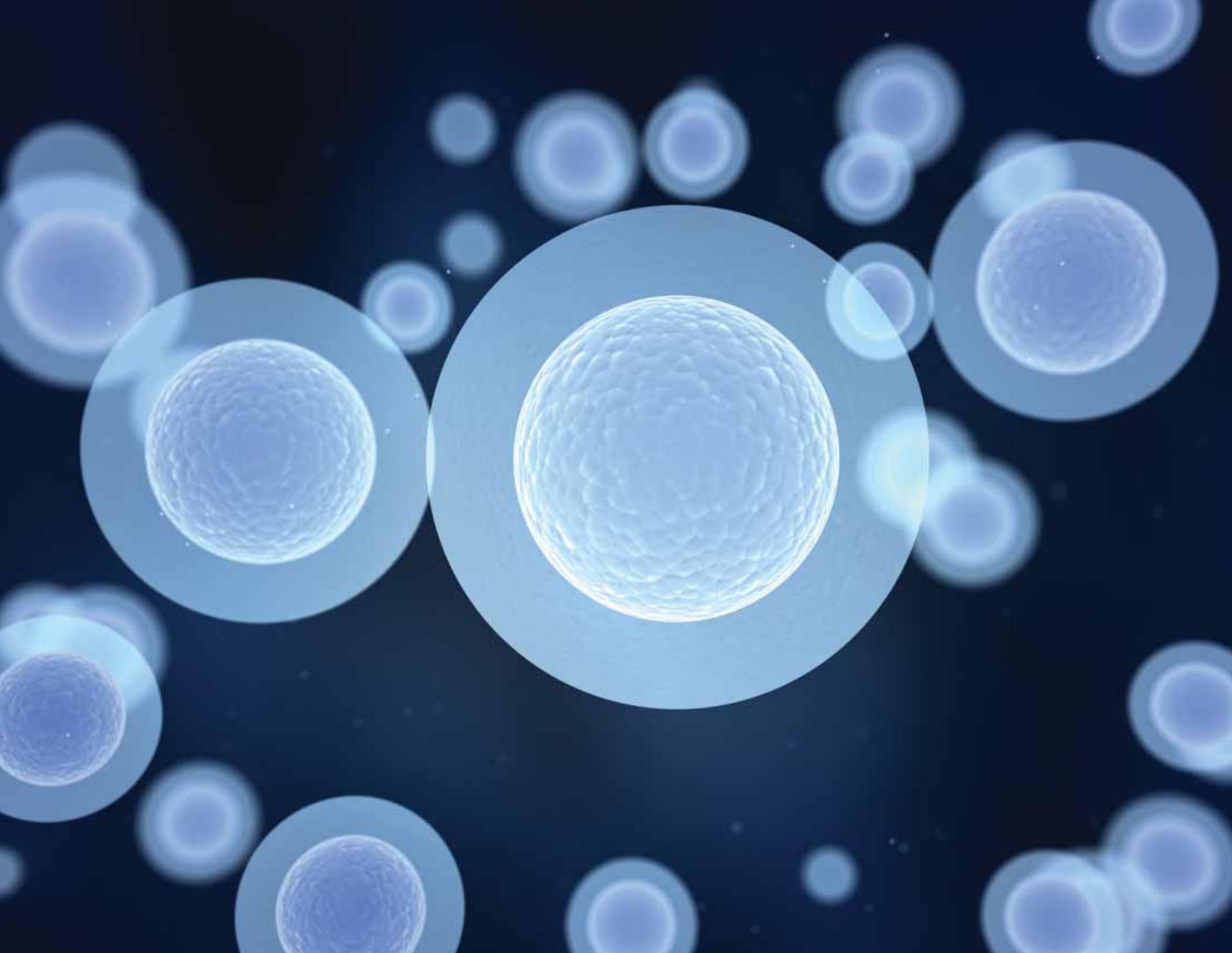


Figure 4. Transfection of stem cells. (A) H9 ESCs or (B) iPSCs were transfected using Lipofectamine 3000 reagent. Cells were stained for pluripotency with the SSEA4 antibody, visualized by fluorescence microscopy, and processed using flow cytometry to determine transfection efficiency and SSEA4⁺ cells.



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.

The natural repair mechanisms of the cell heal the break by either homologous recombination (HR) or non-homologous end-joining (NHEJ). HR is more precise since it requires a template, allowing the introduction of foreign DNA into the target gene. 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](https://www.thermofisher.com/geneengineeringebook)
- Access the genome editing selection guide at [thermofisher.com/geneengineeringguide](https://www.thermofisher.com/geneengineeringguide)
- Find all the genome editing support you need at [thermofisher.com/genomeedit](https://www.thermofisher.com/genomeedit)

Table 3.
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	<ul style="list-style-type: none"> • Easy-to-design genome engineering system • All-in-one plasmid expressing Cas9 and gRNA • Contains reporter system for enriching transfected population 	<ul style="list-style-type: none"> • Multiplexing and screening capable • No cell-specific promoter constraint • No random integration concern 	<ul style="list-style-type: none"> • Multiplexing and screening capable • Superior cleavage efficiency • Fast turnover in cells limits nonspecific cutting • No cell-specific promoter constraint • No random integration concern 	<ul style="list-style-type: none"> • Infect dividing and nondividing mammalian cells • Provide long-term expression of CRISPR gRNA • Loss-of-function screening 	<ul style="list-style-type: none"> • The only provider of TALEN technology that includes the rights under foundational TAL IP • Precise • Flexible; no design restrictions
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	N/A	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

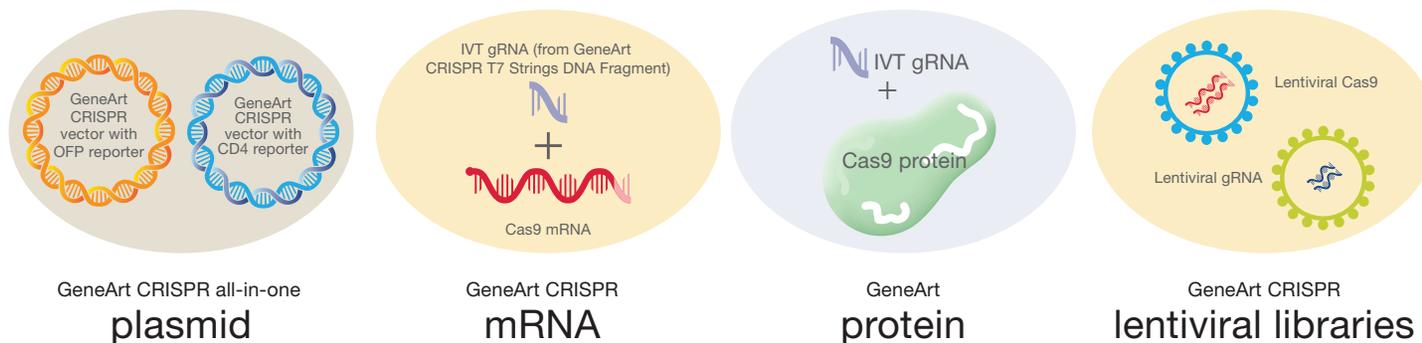
Revolutionizing the field of genome editing

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 (Figure 5). Based on your research needs, you can choose from our different formats of CRISPR tools: CRISPR-Cas9 all-in-one expression plasmids, Cas9 mRNA, Cas9 protein, or CRISPR lentiviral 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



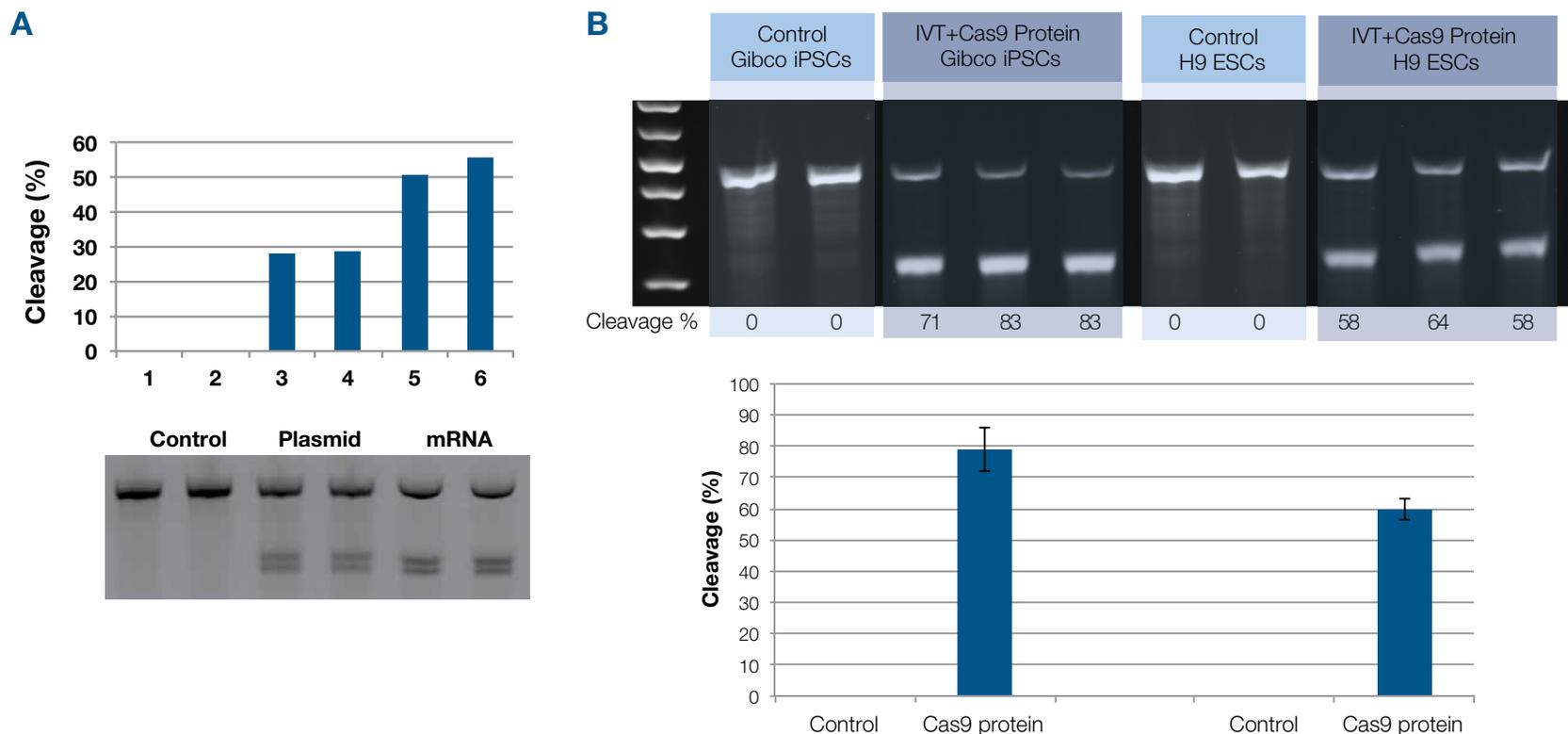


Figure 5. Transfection and electroporation of Invitrogen™ GeneArt™ CRISPR Nuclease Vector with OFP reporter and GeneArt™ CRISPR Nuclease mRNA in mouse ESCs. (A) DNA transfection was performed using Lipofectamine 3000 and RNA with Lipofectamine MessengerMAX reagent, and cells were assayed for genomic cleavage 48 hours after transfection. (B) iPSCs or H9 ESCs were transfected with Cas9 protein formats using the Neon transfection system: Results shown here are for HPRT loci from triplicate samples using 10 µl tips. Highest genomic cleavage was achieved using 1,400 V, 30 ms pulse width, and 1 pulse. Per well: used 1.4 µg of Cas9 protein and 300 ng *in vitro* transcribed gRNA and 1 x 10⁵ cells. We see highest cleavage efficiencies across broad cell types using Cas9 protein format and IVT gRNA.

Characterization tools for gene editing

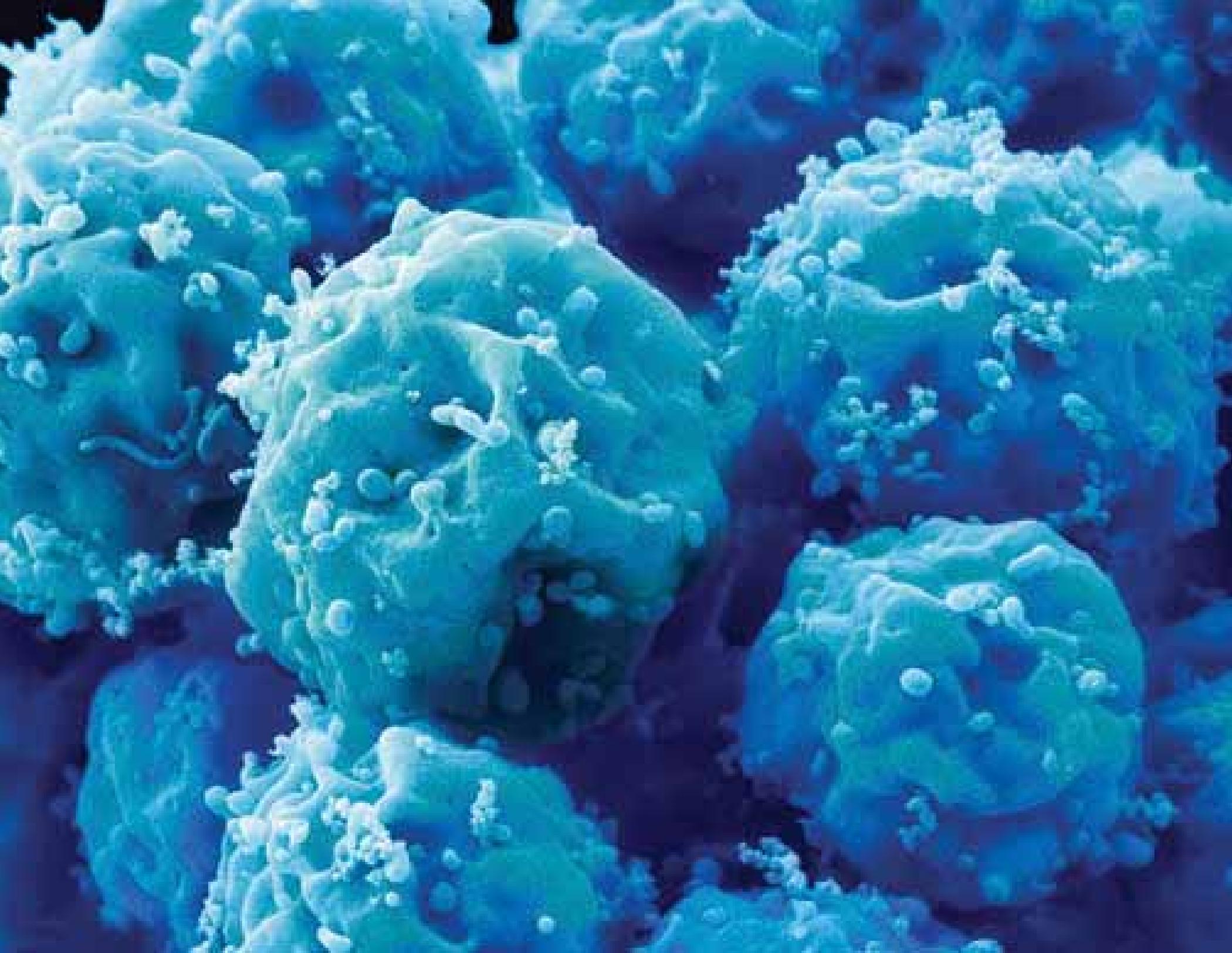
GeneArt Genomic Cleavage Detection Kit

Direct detection of locus-specific genomic DNA modification.

GeneArt Genomic Cleavage Selection Kit

Quick screening of the cleavage activity and enrichment of nuclease-modified cells.

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



Pluripotent stem cell culture

We recognize and understand the preparation that goes into generating pluripotent stem cells (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/pssculture to find the right PSC media for your research.

SUPPORT RESOURCES:

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

Table 5.
Media systems for pluripotent stem cell 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.	10828-010* 10828-028*	A1000701	thermofisher.com/essential8media	12618-012
Recommended substrate	Mouse (ICR) Inactivated Embryonic Fibroblasts	Gibco™ Geltrex™ hESC-qualified Ready-To-Use Reduced Growth Factor Basement Membran 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.

* 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.

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 pluripotent stem cells, 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 pluripotent stem cell culture, Essential 8 Medium helps minimize variability in culture.

The 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



Embryoid body (EB) formation and differentiation
Essential 6™ Medium
Enables more efficient EB formation and differentiation



Stressful events
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

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 truly 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

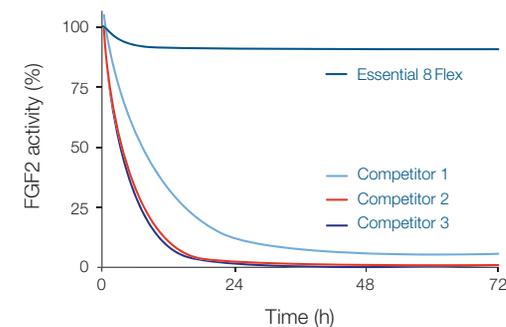
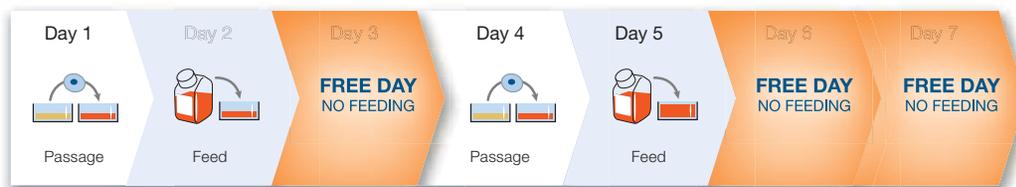
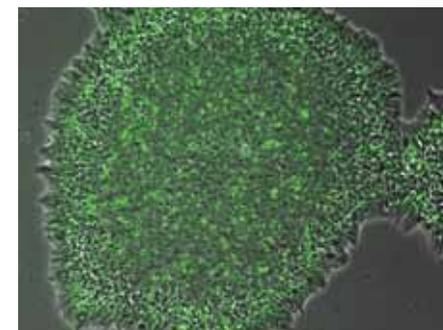


Figure 6. FGF2 activity over time in PSC culture medium. Unlike other feeder-free PSC culture media, Essential 8 Flex Medium has been optimized to extend the activity of unstable components such as FGF2. Extended FGF2 activity allows for routine culture without daily feeding.

PSC culture matrices

We provide extracellular matrices (ECMs) that are designed to minimize adaptation time, maximize cell performance, and help meet regulatory requirements.

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 pluripotent stem cells 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	Optimal for clinical research applications that require human origin materials only
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. A15696-01	Vitronectin (VTN-N) Recombinant Human Protein, Truncated Cat. No. A14700	CTS CELLstart Substrate Cat. No. A10142-01
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	Gibco CTS Knockout SR XenoFree
Source origin	Cultured mouse EHS tumor cells; undefined	Cultured mouse EHS tumor cells; undefined	Human (recombinant); defined	Blend of human native proteins; 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](https://www.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](https://www.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 pluripotent stem cells	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	✓	✓	✓
Recovery component included	✓		
CTS product available	N/A	Gibco™ CTS™ Synth-a-Freeze Cryopreservation Medium Cat. No. A13713-01	N/A
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 induced pluripotent stem cell (iPSC) research. From Alkaline Phosphatase Live Stain, which provides quick verification of pluripotency, to Applied Biosystems™ TaqMan™ hPSC Scorecard™ Panel, which evaluates pluripotency and confirms trilineage differentiation potential, we have the tools you need to characterize with confidence.

Visit [thermofisher.com/characterization](https://www.thermofisher.com/characterization) to find the right characterization tool 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 iPSC cells)	Medium (profiles expression of human PSC and tissue makers)	High (profiles expression of 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	N/A	N/A

TaqMan hPSC Scorecard Panel

Quantitative analysis of trilineage differentiation potential

The TaqMan hPSC Scorecard Panel assesses pluripotency and 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 [1]. The accompanying analysis software gives researchers anywhere in the world the ability to compare the gene expression profiles of their cell lines to a common reference set.

Visit thermofisher.com/scorecard to learn more about this innovative technology.

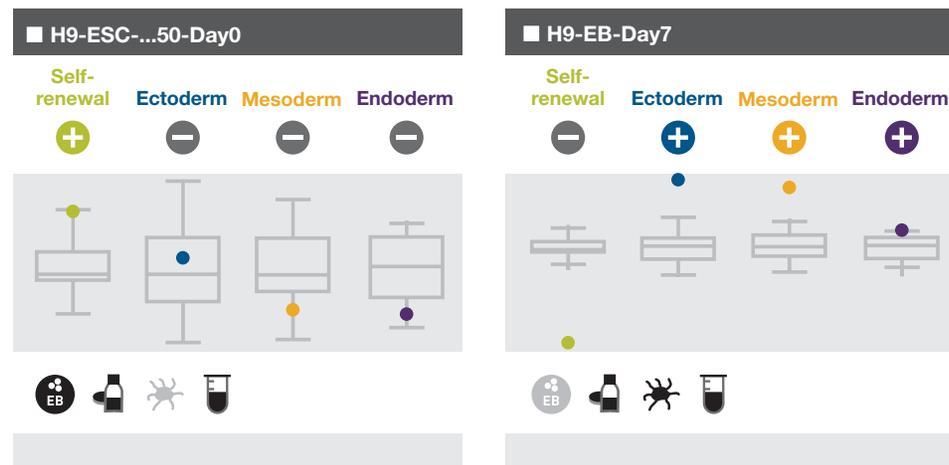


Figure 7. Gene expression results for self-renewal and germ layer markers are summarized in an easy-to-read format.

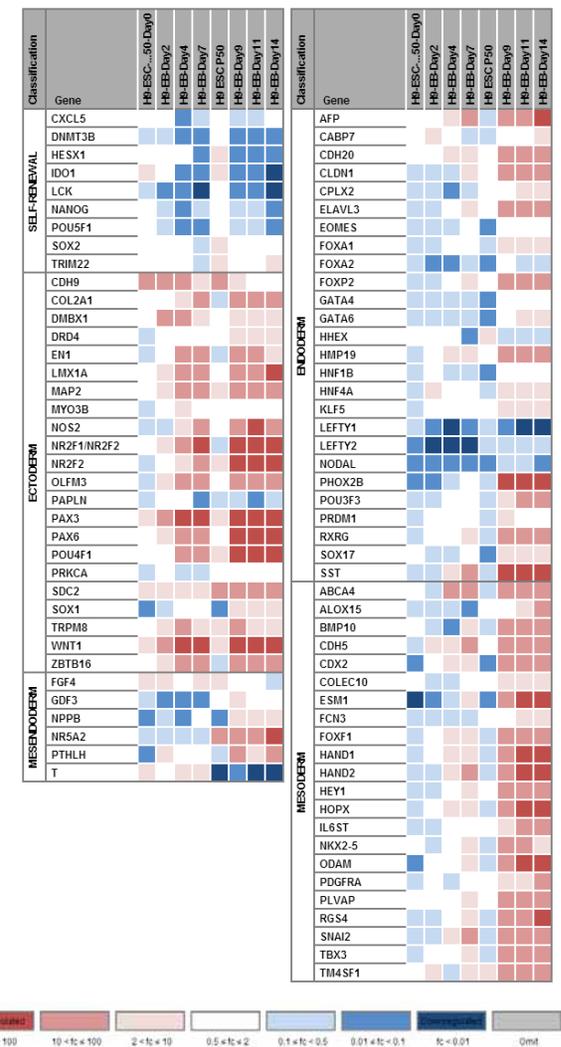
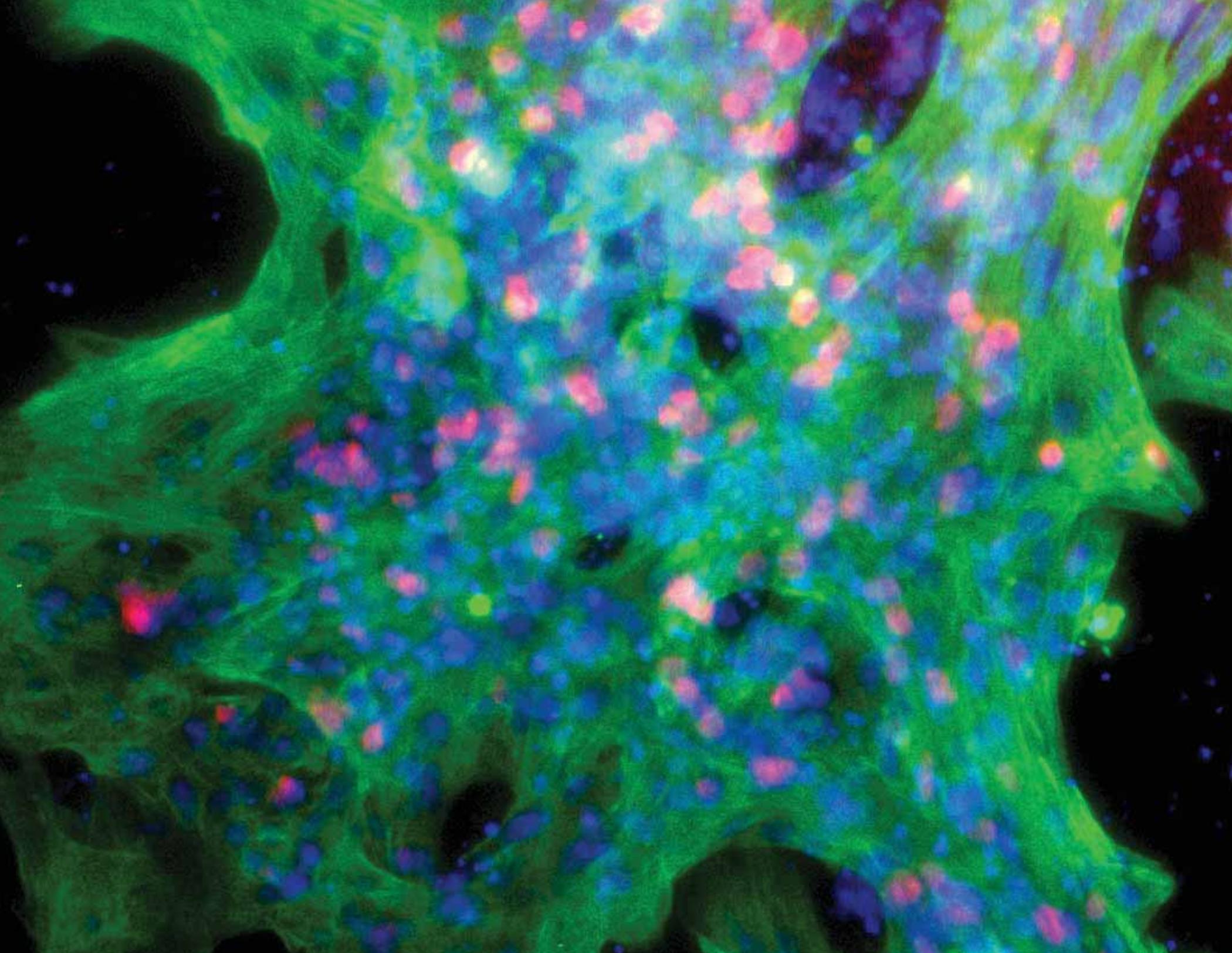


Figure 8. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.



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](https://www.thermofisher.com/stemcellprotocols)
- View complete differentiation portfolio at [thermofisher.com/differentiation](https://www.thermofisher.com/differentiation)

Table 9.
Media systems and reagents for differentiation.

	Ectoderm	Mesoderm	Endoderm
Application	NSC differentiation	Cardiomyocyte differentiation	Definitive endoderm differentiation
Media system	Gibco™ PSC Neural Induction Medium Cat. No. A1647801	Gibco™ PSC Cardiomyocyte Differentiation Kit Cat. No. A25042-SA	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	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	14 days	2 days
Cell type generated	Neural stem cells	Cardiomyocytes	Definitive endoderm
Media format	50X supplement/500 mL basal, 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 pluripotent stem cells (Figure 9) 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 10). 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 11).

For more information, go to thermofisher.com/nscdiff



Human NSC Immunocytochemistry Kit

The Invitrogen™ Molecular Probes™ 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/psccimmunokits

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 multiplexed images with minimal effort
- Save time and avoid cell loss by eliminating unnecessary wash steps in your staining protocol

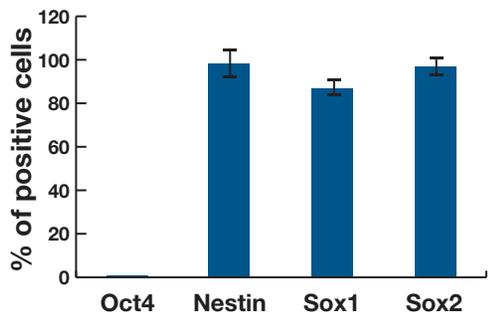
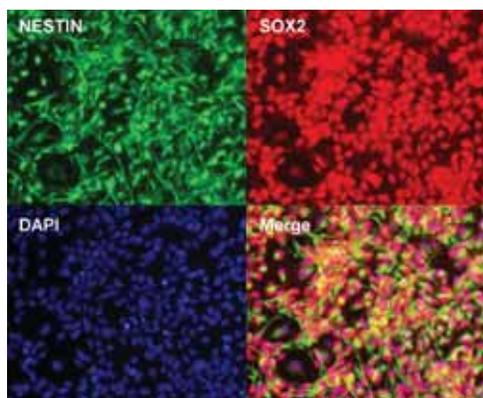
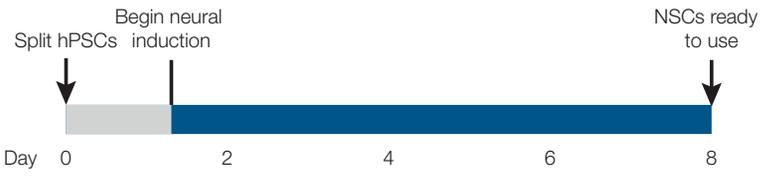


Figure 9. 80–90% neural induction efficiency.

Gibco PSC Neural Induction Medium



Other methods of generating NSC

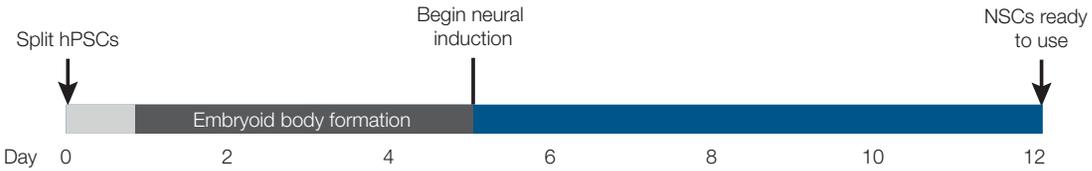


Figure 10. Unlike existing methodologies, Gibco PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation which adds time, labor, and variability.

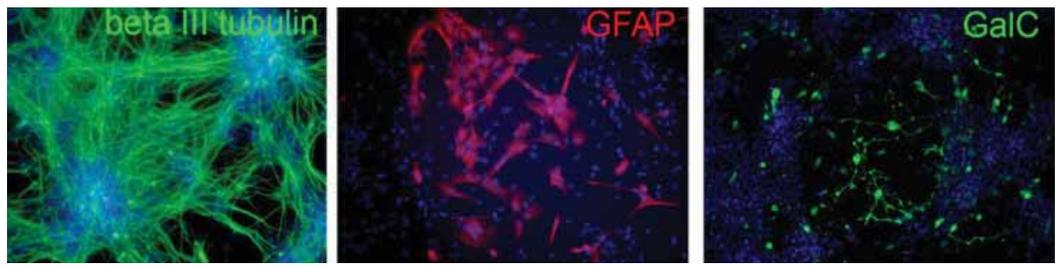


Figure 11. Neural Stem Cells (NSCs) generated using the Gibco PSC Neural Induction Medium have high expression of NSC markers and can be further differentiated into other neural cell types.

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 pluripotent stem cells to contracting cardiomyocytes in as few as 8 days (Figure 12). 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 (Figure 13), resulting in functional cardiomyocytes that express relevant physiological markers, contract in culture, and can be subsequently maintained in culture for more than 15 days.



Human Cardiomyocyte Immunocytochemistry Kit

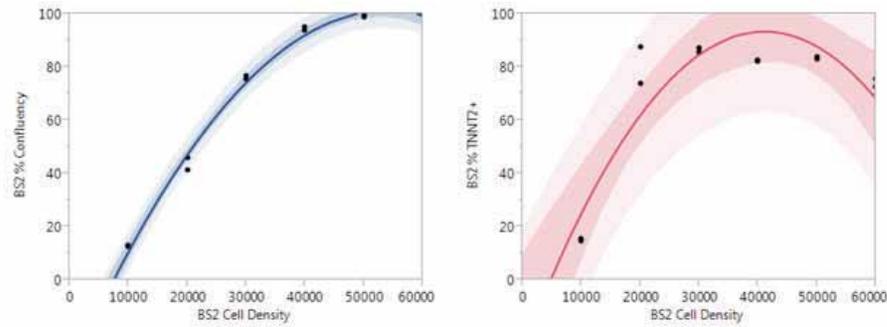
The Invitrogen™ Molecular Probes™ Human Cardiomyocyte Immunocytochemistry Kit enables optimal image-based analysis of 2 key cardiomyocyte markers: NKX2.5 and TNNT2/cTnT. The kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and all the premade buffers for an optimized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

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

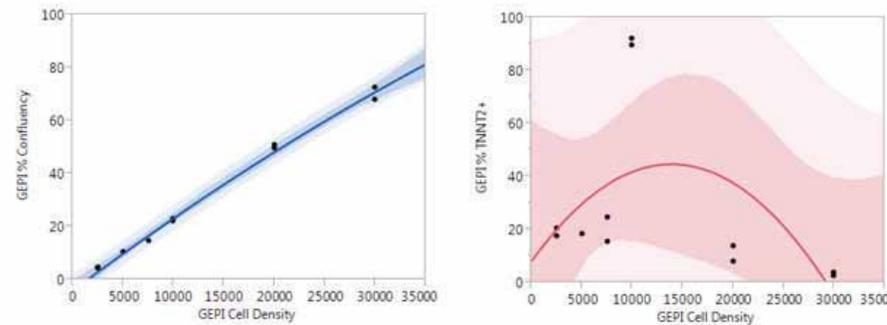
With this kit, you can:

- 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 multiplexed 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

iPSC
(CytoTune/
Sendai virus
reprogramming)



**Gibco™ Human
Episomal iPSC
Line**
(Episomal vector
reprogramming)



H9 hESC

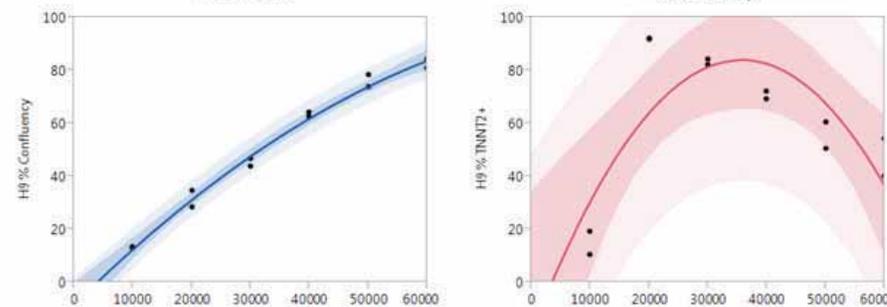


Figure 12. Efficiency across multiple PSC lines. Seeding density is crucial for optimal PSC cardiomyocyte differentiation. TrypLE™-dissociated PSC lines were used for setup of these studies. For two CytoTune™-derived lines, BS2 iPSC was observed to be promiscuous at higher density. For Gibco Human Episomal iPSC Line, it was also found to be optimal at a specific density. For hESC, H9 was observed to be promiscuous at various densities. JMP™ Profiler tool identified optimal seeding density for efficient differentiation of different PSC lines.

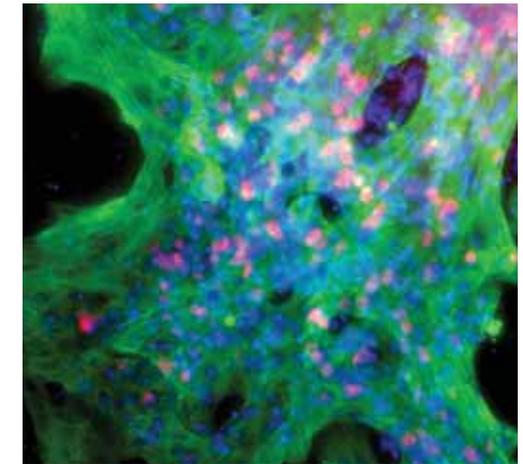


Figure 13. iPSCs were differentiated for 14 days using the PSC Cardiomyocyte Differentiation Kit. The cells were stained using the Human Cardiomyocyte Immunocytochemistry Kit for the following markers: NKX2.5 (red) for early cardio mesoderm and TNNT2/cTnT (green) for cardiomyocytes along with DAPI nuclear DNA counterstaining (blue).

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 (Figure 14). 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 α ⁻ definitive endoderm cells with only 2 components in just 2 days (Figure 15).

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 16) and are capable of differentiating to downstream lineages.

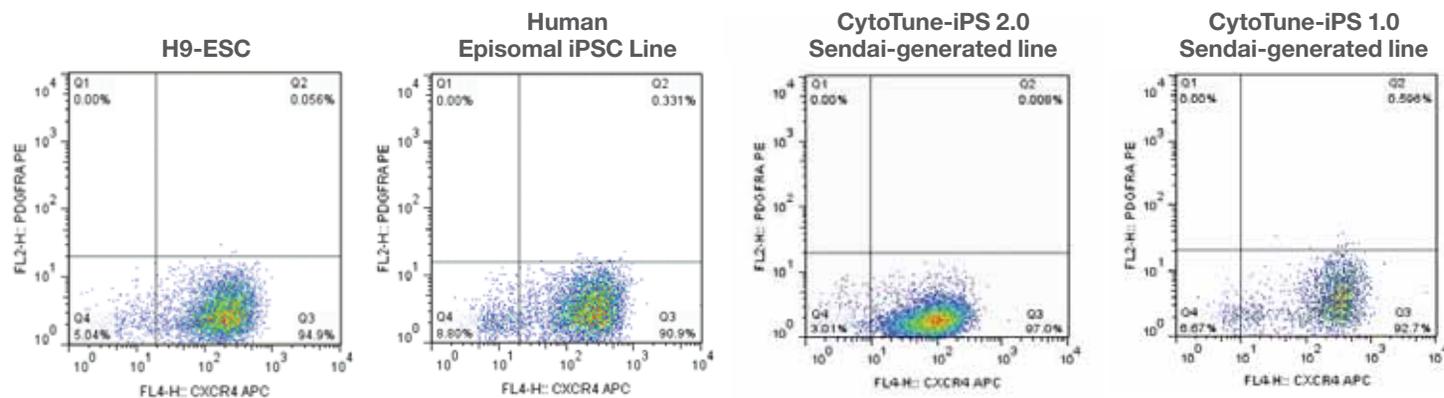
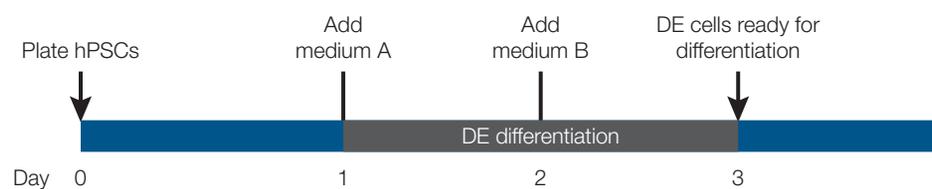


Figure 14. The PSC Definitive Endoderm Induction Kit produces DE populations with high efficiency ($\geq 90\%$) across hESC and iPSC cell lines, including cell lines reprogrammed using episomal vectors or CytoTune™ kits. Representative dot plots show CXCR4⁺/PDGFR α ⁻ cell populations derived from various cell lines. For each experiment, unstained cells were used to set quadrant gates.

PSC Definitive Endoderm Induction Kit



STEMdiff™ Definitive Endoderm Kit*

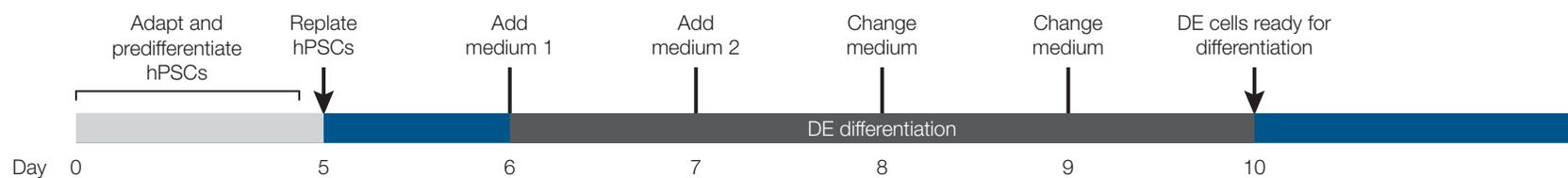
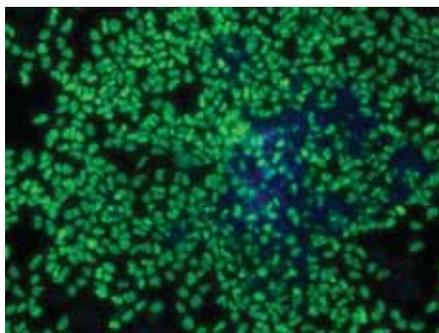
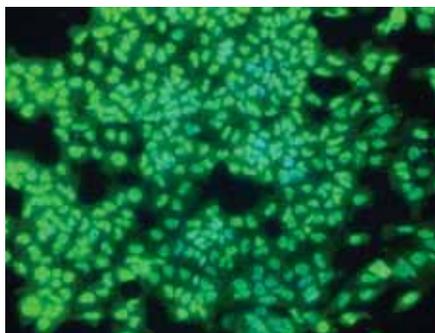


Figure 15. Compared to other differentiation protocols,* the PSC Definitive Endoderm Induction Kit produces cells in up to 50% less time and requires no predifferentiation or mixing of media.

SOX17



FOXA2



Oct4

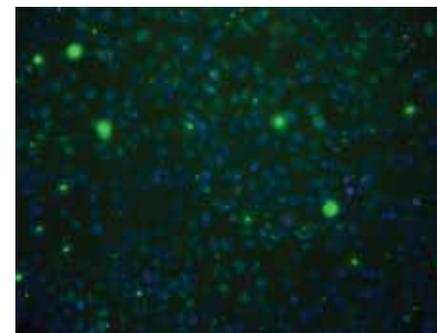
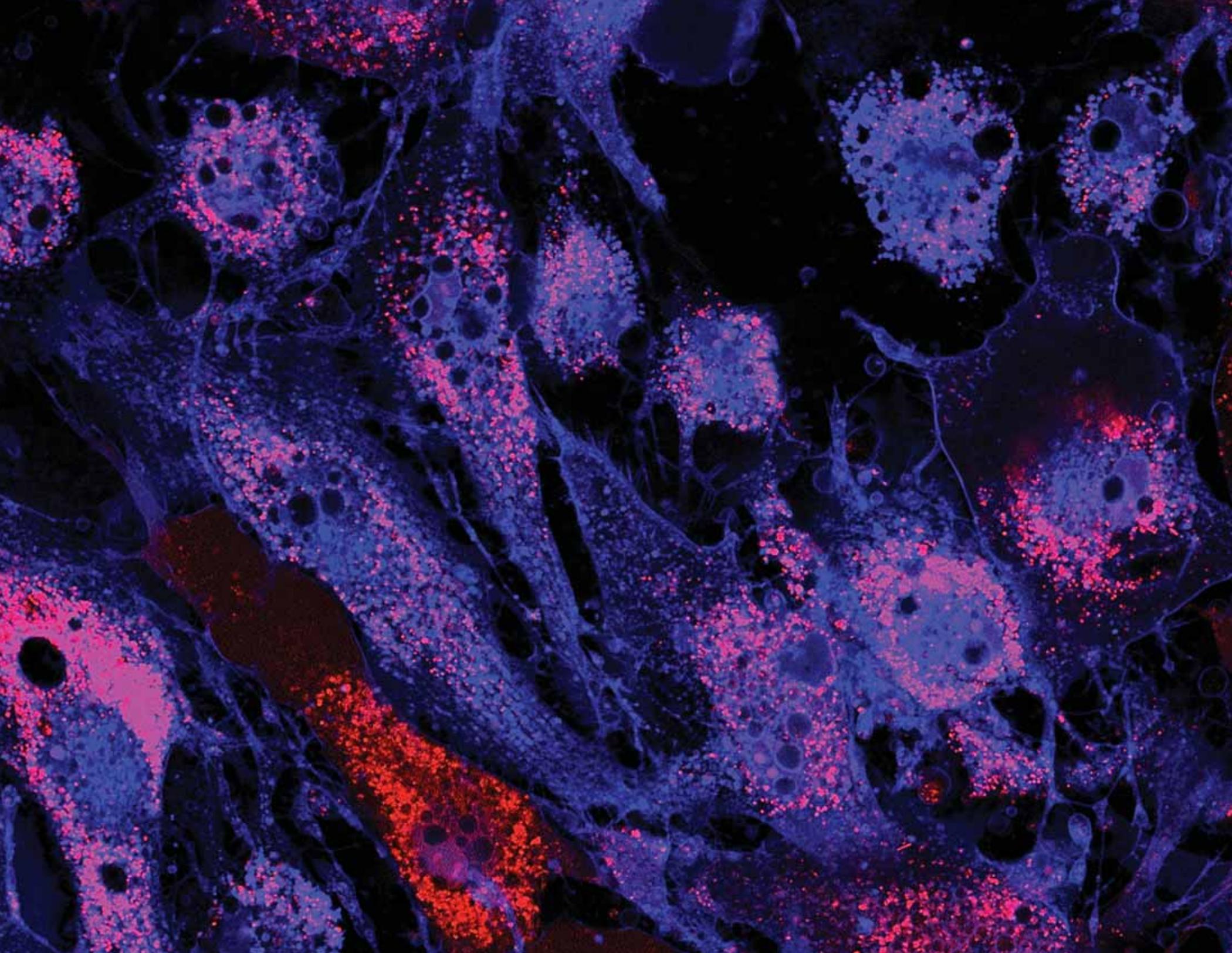


Figure 16. Immunocytochemistry of hESCs treated with the PSC Definitive Endoderm Induction Kit. At day 3, induced cells were immunostained for the endodermal transcription factors SOX17 and FOXA2, and the pluripotent marker Oct4. Nuclei were counterstained with DAPI (blue) to assess total cell numbers.



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.

- Qdot™ nanocrystals
- Alexa Fluor™ dyes
- Alexa Fluor™ secondary antibodies and streptavidin
- Primary antibodies
- Alkaline Phosphatase Live Stain
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- Applied Biosystems™ protein expression sample preparation kits
- Ambion™ RNA extraction and purification kits
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With fluorescence capabilities—bright-field and 2 optional fluorescence channels—the Countess™ II FL can count cells, monitor fluorescent protein expression, and measure cell viability. Designed with flexibility in mind, the Countess II FL can also be configured to use a full range of light cubes that provide more than 15 fluorescence color options. For a virtual demo, go to thermofisher.com/countess



Neon Transfection System

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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 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



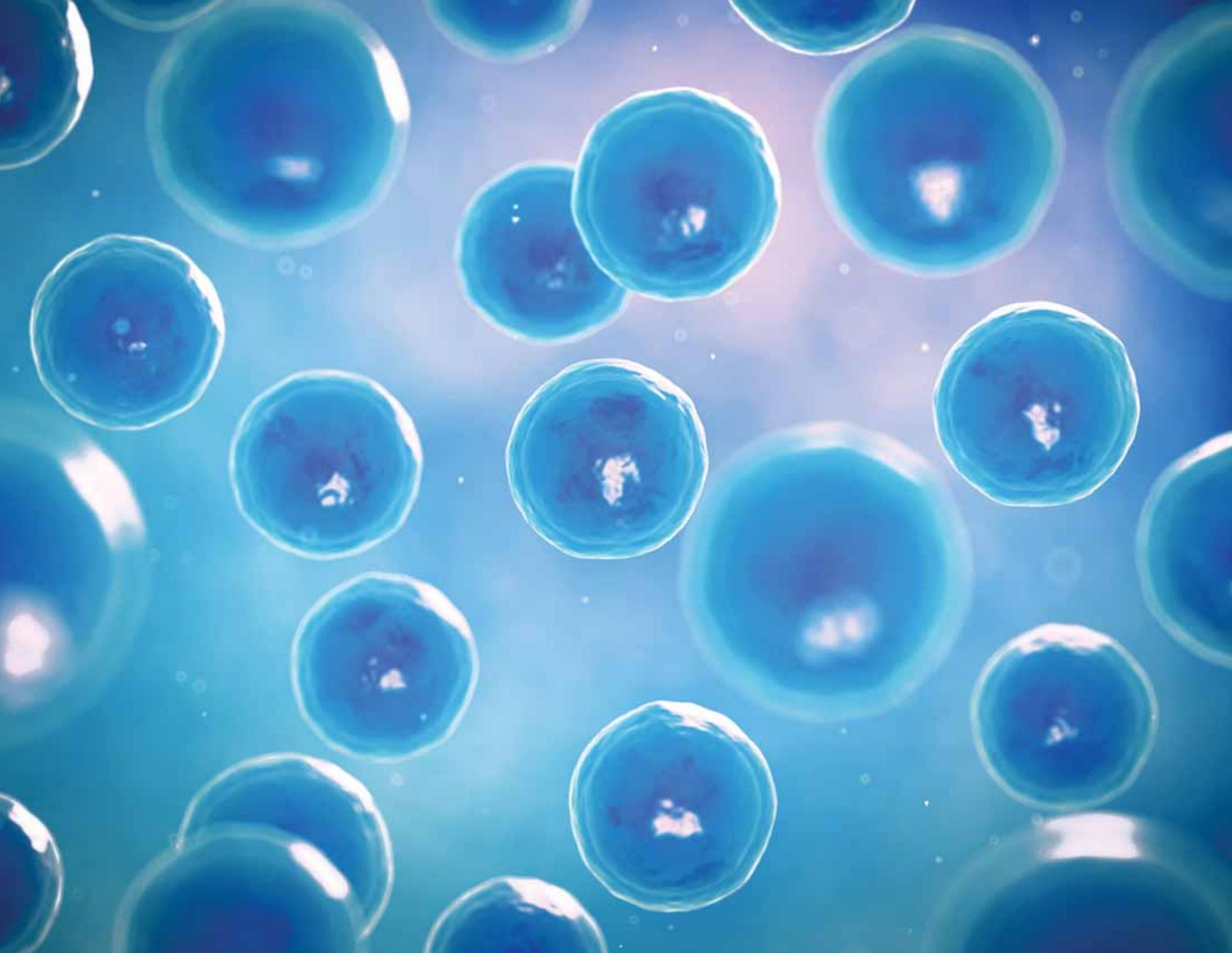
Ion Personal Genome Machine (PGM) sequencer

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Ion Proton System

The Ion Proton semiconductor sequencing system offers high quality, affordable human transcriptome, exome, and genome sequencing. View the technology behind the Ion Proton™ System at thermofisher.com/proton



CellModel Services

Built on the stem cell innovations that we have introduced throughout the past decade, our CellModel™ Services enable stem cell scientists to reach their desired outcomes faster. We offer stem cell researchers choices at every stage of their research—innovative tools that make it easier for you to “do it yourself” and 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.

- **Reprogramming**—human fibroblasts or blood cells reprogrammed in 4 to 6 months with top clones expanded, cryopreserved, and characterized.
- **Differentiation**—pluripotent stem cells differentiated into neural stem cells and terminal lineages.
- **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 HTS capabilities.

Experienced service providers

CellModel Services are delivered by scientists from our Custom Biology team who have helped researchers with their cellular engineering and custom assay development projects for years. 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.

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Stem cell support resources

Get the stem cell support you need—the way you want it. Stem cell research can be time-consuming, finding support shouldn't be. That's why we give you easy access to stem cell resources. It's all about getting you everything you need, and providing it in a format that fits your learning preference.

Find the support you're looking for at thermofisher.com/stemcellsupport



Logical
(mathematical)

Reasoning and orderly systems are the way your mind works. Visit our protocol portal for access to over 70 experimental protocols and FAQs, such as:

- Generation of hiPSCs from peripheral mononuclear blood cells with CytoTune-iPS Sendai Reprogramming Kit
- Episomal iPSC reprogramming vectors FAQs
- Culturing human neural stem cells



Verbal
(linguistic)

You respect the power of words, both in speech and writing. We know the value of proven experiments and provide access to lists of publications that use some of our market-leading products:

- Publications citing Essential 8 Medium and vitronectin
- KnockOut Serum Replacement publications list
- Neural Cell Culture Publication Finder



Visual or Aural
(spatial or auditory-musical)

Visual learners prefer pictures, images, and spatial understanding, while aural learners prefer sound and music. We offer how-to videos to help ensure experiment set-up success, and interviews and seminars from leaders in the stem cell field:

- How to prepare Essential 8™ Medium for pluripotent stem cell culture
- Dr. Jeanne Loring interview (video)—*Inducing Excitement with Pluripotency*
- Dr. Rupa Shevde seminar (video)—*The Evolution of Culture Media*



Social
(interpersonal)

Learning in groups or with other people works well for you. Technical support specialists are standing by via phone, email, and live chat to help walk you through your stem cell challenges.

Stem cell technical support contacts:

Email: stemcell-help@lifetech.com

Phone: 800.955.6288

Follow us on Twitter: @LifeTechSupport



Physical
(kinesthetic)

You prefer using your body, hands, and sense of touch. Learn in person with our hands-on stem cell training. Classes provide a comfortable learning experience and allow for personalized interactions between trainers and attendees.

For workshop dates and locations, go to thermofisher.com/lifelab

Stem cell training

We have proudly established LifeLab™ 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 are 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 at thermofisher.com/lifelab

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.



Ordering information

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StemPro Human Adipose-Derived Stem Cell Kit	R7788110
StemPro BM Mesenchymal Stem Cells	A15652
StemPro MSC SFM XenoFree	A10675-01
StemPro Neural Stem Cells	A15654
Human Dermal Fibroblasts, Adult	C-013-5C
StemPro CD34+ Cell Kit	A14059
StemPro-34 SFM	10639-011
CTS Immune Cell Serum Replacement*	A25961-01
StemPro NSC SFM	A1050901
Reprogramming	
Episomal iPSC Reprogramming Vectors	A14703
Epi5 Episomal iPSC Reprogramming Vectors	A15960
CytoTune-iPS 2.0 Sendai Reprogramming Kit (1 pack)	A16517
CytoTune-iPS 2.0 Sendai Reprogramming Kit (3 pack)	A16518
Transfection	
Lipofectamine 3000 Transfection Reagent	L3000-001
Lipofectamine RNAiMAX Transfection Reagent	13778-075
Lipofectamine MessengerMAX Transfection Reagent	LMRNA015
ExpiFectamine 293 Transfection Kit	A14526
Lipofectamine 2000 Transfection Reagent	11668-019
InvivoFectamine 3.0 Reagent	IVF3001

Product	Cat. No.
Genome editing	
GeneArt CRISPR Nuclease mRNA	A25640
GeneArt CRISPR Nuclease Vector with GFP Reporter Kit	A21174
GeneArt CRISPR Nuclease Vector with CD4 Enrichment Kit	A21175
GeneArt CRISPR U6 Strings DNA	Contact geneartsupport@ thermofisher.com
GeneArt CRISPR T7 Strings DNA	Contact geneartsupport@ thermofisher.com
GeneArt TALs	Contact geneartsupport@ thermofisher.com
Culture	
KnockOut Serum Replacement**	10828-028
StemPro hESC SFM	A10007-01
Essential 8 Medium	A1517001
Essential 6 Medium	A1516401
Essential 8 Flex Medium	A2858501
RevitaCell Supplement	A26445-01
Collagenase IV	17104-019
StemPro EZPassage Disposable Stem Cell Passaging Tool	23181-010
CTS TrypLE Select Enzyme	A12859-01

Product	Cat. No.
Matrices	
CTS CELLstart Substrate	A10142-01
Geltrex hESC-qualified Ready-To-Use Reduced Growth Factor Basement Membrane Matrix	A15696-01
Geltrex LDEV-Free hESC Qualified Reduced Growth Factor Basement Membrane Matrix	A1413301
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Cryopreservation	
PSC Cryopreservation Kit	A26446-01
Synth-a-Freeze Cryopreservation Medium	A12542-01
CTS Synth-a-Freeze Cryopreservation Medium	A13713-01
Recovery Cell Culture Freezing Medium	12648-010
RevitaCell Supplement	A26445-01
Differentiation	
PSC Neural Induction Medium	A1647801
PSC Cardiomyocyte Differentiation Kit	A25042-SA
PSC Definitive Endoderm Induction Kit	A27654-SA

Product	Cat. No.
Characterization	
Alkaline Phosphatase Live Stain	A14353
TaqMan Human Stem Cell Pluripotency Array	4385344
TaqMan hPSC Scorecard Panel, Fast 96-well	A15876
TaqMan hPSC Scorecard Panel, 384-well	A15870
Human Cardiomyocyte Immunocytochemistry Kit	A25973
Human Neural Stem Cell Immunocytochemistry Kit	A24354
Pluripotent Stem Cell 4-marker Immunocytochemistry Kit	A24881
3-Germ Layer Immunocytochemistry Kit	A25538
PSC Immunocytochemistry Kit (OCT4, SSEA4)	A25526
PSC Immunocytochemistry Kit (SOX2, TRA-1-60)	A25525
AlexaFluor™ 488 CD44 Live Cell Imaging Kit	A25528
AlexaFluor™ 488 Tra-1-60 Live Cell Imaging Kit	A25618
AlexaFluor™ 555 Tra-1-60 Live Cell Imaging Kit	A24879
AlexaFluor™ 594 Tra-1-60 Live Cell Imaging Kit	A24882

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Not for use in diagnostic procedures.

*For *In Vitro* Diagnostic Use

**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.



Reference

1. Bock C, Kiskinis E, Versteppen 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.

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