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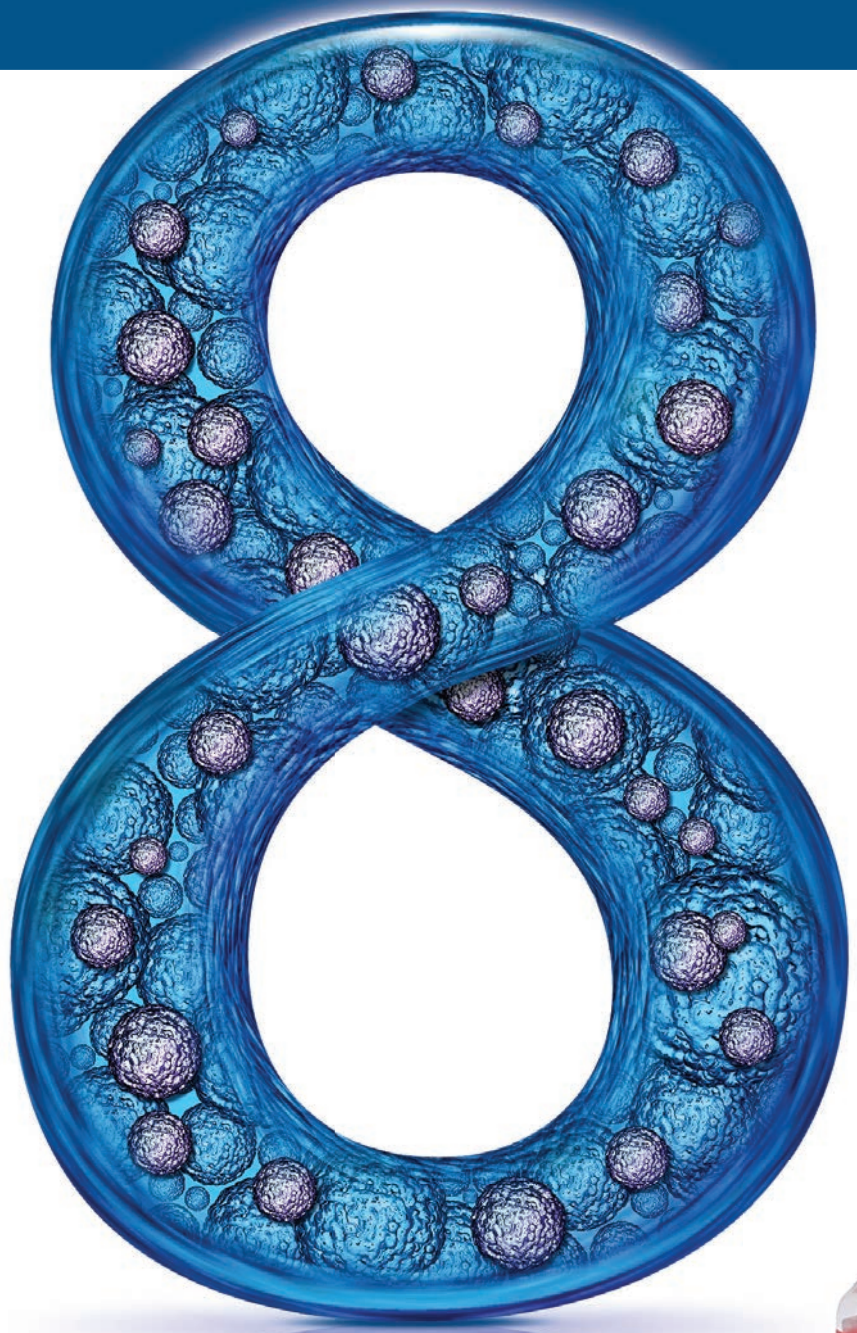


A simple choice

Essential 8 media systems

ThermoFisher
SCIENTIFIC

Your stem cells thrive with the essentials

Gibco™ Essential 8™ Medium is a feeder-free, xeno-free medium that was 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 is manufactured under cGMP conditions.

> "Essential 8 Medium does exactly what you need it to do. And because it is so simple, it can be made in a more controlled way."
>
> — Emile Nuwaysir, PhD, Cellular Dynamics International

Just what cells need, nothing they don't

Many feeder-free stem cell media contain 20 or more components in their formulations (Table 1). While these media may adequately grow and maintain PSCs, they also contain many variables and commonly exhibit lot-to-lot inconsistencies.

By removing highly undefined proteins (such as BSA) and other components, and including only the ingredients necessary for PSC culture, Essential 8 Medium helps minimize variability in culture. Additionally, all media within the Essential 8 family are manufactured according to cGMP conditions—a further safeguard against lot-to-lot variability that minimizes the risk of variable outcomes and unintended cell differentiation.

Table 1. A comparison of published formulations of STEMCELL™ Technologies mTeSR™ medium and Essential 8 Medium shows significant differences in the number of components required to support PSC growth and expansion.

Components	mTeSR	Essential 8
DMEM/F12	X	X
L-ascorbic acid	X	X
Selenium	X	X
Transferrin	X	X
$NaHCO_3$	X	X
Insulin	X	X
FGF2	X	X
TGFB1	X	X
Albumin (BSA)	X	
Glutathione	X	
L-glutamine	X	
Defined lipids	X	
Thiamine	X	
Trace elements B	X	
Trace elements C	X	
β-mercaptoethanol	X	
Pipecolic acid	X	
LiCl	X	
GABA	X	
H_2O	X	

Table 2. Gibco™ Essential media selection guide.

Product	Essential 6 Medium	Essential 8 Medium	Essential 8 Flex Medium	CTS Essential 8 Medium
Ideal for	PSC applications requiring customization of growth factors (e.g., differentiation, low–growth factor PSC culture)	PSC culture with everyday feeding schedule	PSC culture with flexible feeding schedule	PSC culture in translational research or clinical applications
Contains TGFB1 and FGF2	No	Yes	Yes	Yes
Weekend-free feeding schedule	No	No	Yes	No
Defined	Xeno-free; no animal-derived components; human derived	Xeno-free; no animal-derived components; human derived	Xeno-free; no animal-derived components; human derived	Animal origin–free (AOF); no animal- or human-derived components
Regulatory documentation available	No	No	No	Yes
ISO- and GMP-certified manufacturing standards	Yes	Yes	Yes	Yes
Intended use statement	For Research Use Only	For Research Use Only	For Research Use Only	For Research Use or Manufacturing of Cell-, Gene-, or Tissue-Based Products

Where would you like to gain control?

Choose the Essential medium that's right for you

PSC maintenance and expansion—Essential 8 Medium

Our most defined and consistent feeder-free medium for iPSCs

Determine what goes in and what stays out of your pluripotent stem cell (PSC) cultures with a medium that contains only the 8 essential ingredients required to consistently grow and maintain PSCs, while minimizing variability.

- **Consistent**—reduced variability compared to conventional feeder-free culture media
- **Cost-effective**—economical and scalable PSC culture compared to other feeder-free culture media
- **Robust**—reliable and consistent cultures with a xeno-free, cGMP-defined medium

Clinical applications—Gibco™ CTS™ Essential 8™ Medium

Transition seamlessly from the bench to clinical applications

CTS Essential 8 Medium is the first globally available animal origin–free hPSC culture medium designed to meet international regulatory requirements.

- **Reduce risks**—animal and human origin–free, fully defined
- **Facilitate regulatory filings**—cGMP-manufactured and supported by regulatory documentation, including an FDA Drug Master File
- **Seamless transition**—same 8-component formulation as Research Use Only (RUO) Essential 8 Medium, but with AOF components

Flexible feeding schedule—Gibco™ Essential 8™ Flex Medium

Manage your PSC culture schedule and eliminate daily feeding

Essential 8 Flex Medium is formulated to extend the activity of key heat-sensitive components found in PSC culture, including basic fibroblast growth factor (FGF2), without sacrificing performance.

- **Flexible feeding schedule**—maintains pluripotency over a full 2-day period without feeding cells
- **Maintains pluripotency more consistently**—significantly limits fluctuations in FGF2 levels seen in other feeder-free media
- **Proven**—based on the original Essential 8 Medium formulation; maintains pluripotency and normal karyotypes for >50 passages

Spontaneous or directed differentiation—Gibco™ Essential 6™ Medium

A versatile basal medium for differentiation and other PSC applications

Together with Essential 8 Medium, Essential 6 Medium completes a defined, xeno-free PSC workflow by serving as a growth factor–free base for differentiation and reprogramming.

- **Flexible**—provides a flexible growth factor-free base where TGFβ, bFGF, or other proteins, molecules, and supplements can be added to suit different applications
- **Enables differentiation**—does not contain bFGF, thereby enabling embryoid body (EB) formation and directed differentiation
- **Supports reprogramming**—does not contain TGFβ, which has a negative effect on reprogramming efficiency

Reliable and consistent PSC cultures—Essential 8 Medium

Proven to maintain pluripotency in multiple PSC lines, Essential 8 Medium has been used to support PSC growth for >50 passages with no signs of karyotypic abnormalities and while maintaining the ability of PSCs to differentiate into all three germ line lineages (Figures 1–3).

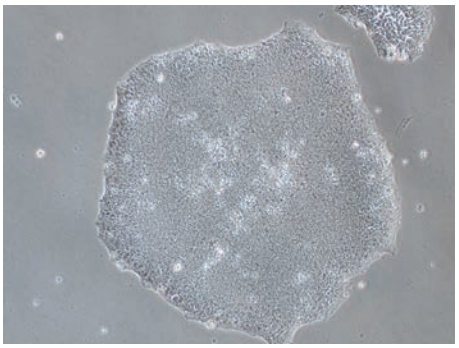
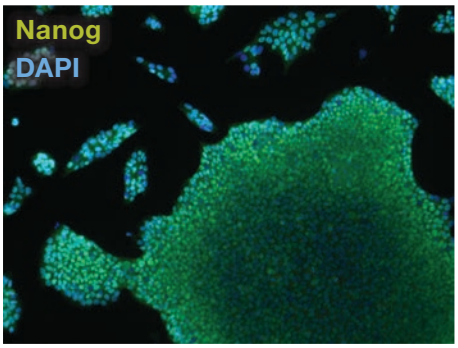


Figure 1. PSC morphology—PSCs cultured in Essential 8 Medium on Gibco™ vitronectin at passage 4.



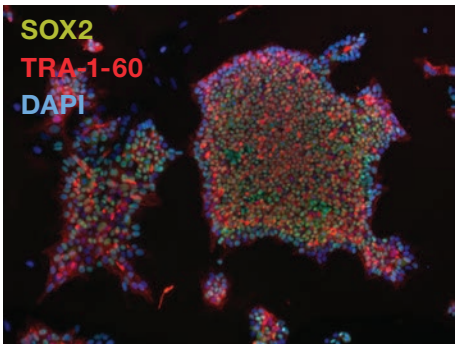
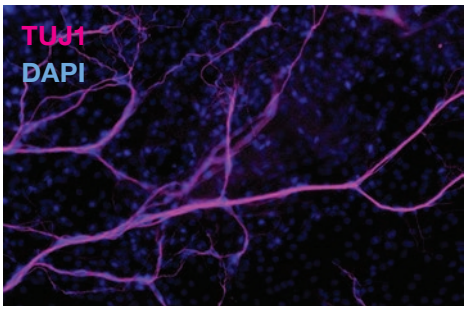
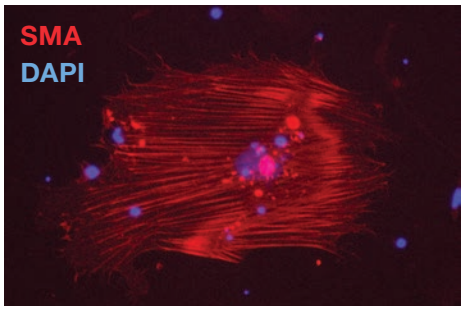


Figure 2. PSC marker staining—Nanog, Tra-1-60, and Sox2 staining of PSCs cultured in Essential 8 Medium on vitronectin-coated plates at passage 50.

Ectoderm



Mesoderm



Endoderm

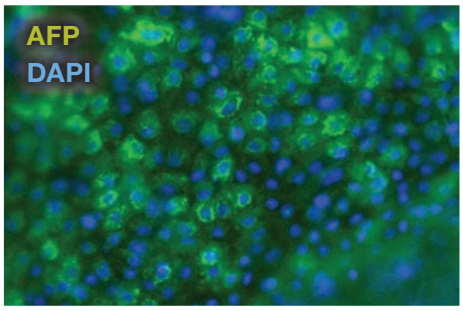


Figure 3. Trilineage differentiation—ectoderm (beta-III-tubulin, TUJ1), mesoderm (smooth muscle actin, SMA), and endoderm (alpha-fetoprotein, AFP) staining of embryoid bodies generated from PSCs cultured in Essential 8 Medium on rhVTN-N–coated plates. DAPI (blue) was used as a counterstain.

Essential 8 Medium has been cited in over 600 peer-reviewed publications.

Flexible feeding schedule with healthy cells and robust differentiation potential—Essential 8 Flex Medium

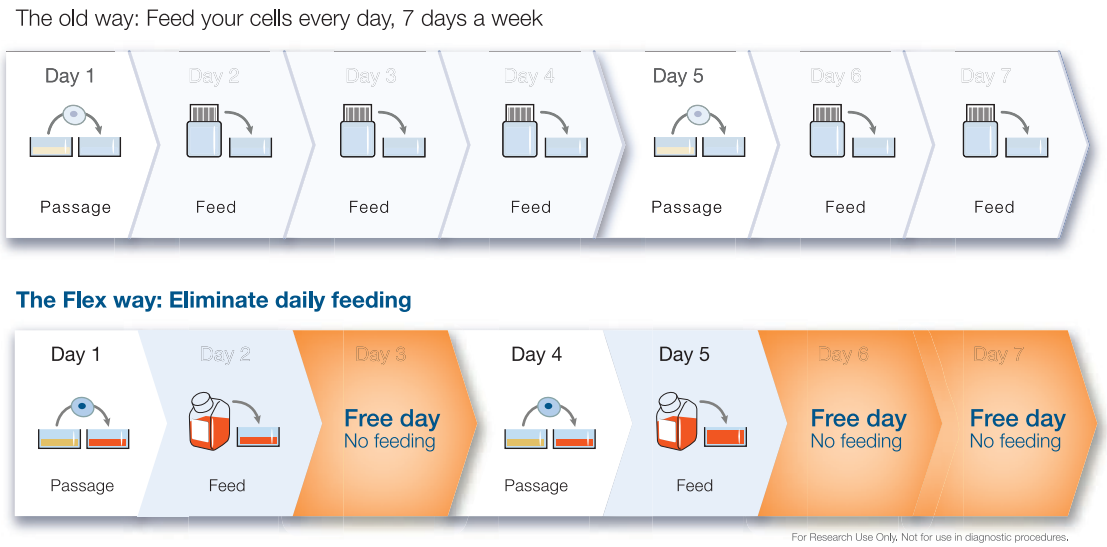


Figure 4. Culture schedule comparison. Unlike other feeder-free media, Essential 8 Flex Medium eliminates the need to manage cultures daily, enabling a truly weekend-free schedule for expansion and maintenance of PSCs. Alternative feeding schedules are available at **thermofisher.com/essential8flex.**

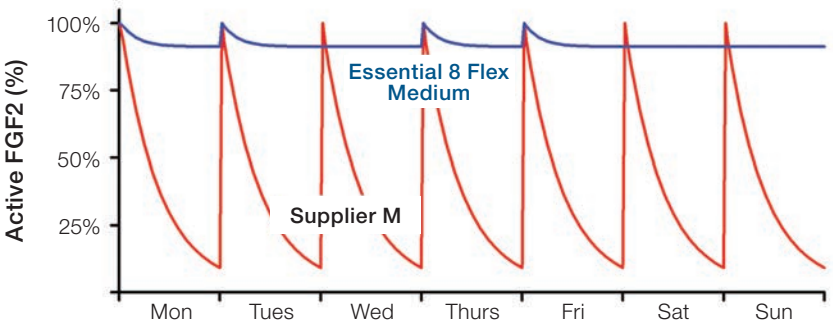


Figure 5. Essential 8 Flex Medium limits fluctuations in levels of active FGF2. Measurements of heat-mediated loss of active FGF2 were used to simulate active FGF2 levels in PSC cultures over the course of a week. Standard PSC culture medium (red curve) cultures were fed on a daily basis, while Essential 8 Flex Medium (blue curves) cultures were fed according to the suggested protocol outlined in Figure 4.

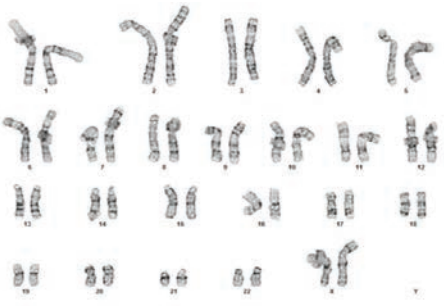


Figure 6. Long-term stability of cells cultured in Essential 8 Flex Medium. Healthy karyotypes have been observed after long-term culture of PSCs in Essential 8 Flex Medium.

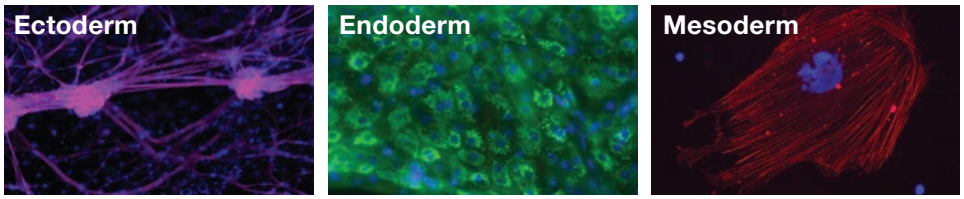


Figure 7. Confirmation of tri-lineage differentiation potential in Essential 8 Flex Medium. The potential for cells from Essential 8 Flex Medium cultures to differentiate into cells of ectoderm, endoderm, and mesoderm lineages is unaffected by long-term culture in Essential 8 Flex Medium. This has been confirmed using both spontaneous differentiation from embryoid bodies and directed differentiation to neural stem cells, cardiomyocytes, and definitive endoderm cells.

Seamless transition from the bench to clinical applications—CTS Essential 8 Medium

CTS Essential 8 Medium has replaced the human-origin components with recombinant proteins, resulting in the first animal origin–free, feeder-free, GMP-manufactured product globally available. It is based on the original Essential 8 Medium formulation and exhibits comparable performance. This provides a seamless transition from early development through clinical translation and beyond.

Long-term maintenance of PSC cultures

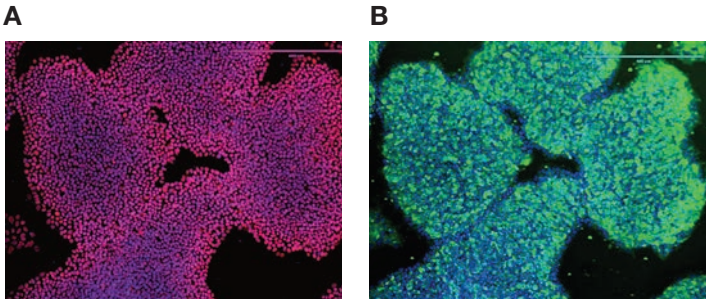


Figure 8. PSC marker expression. PSCs were cultured in CTS Essential 8 Medium for over 30 passages and then stained using the Invitrogen™ PSC 4-Marker Immunocytochemistry Kit. PSCs exhibit strong expression of the PSC markers **(A)** Oct4 (red) and **(B)** SSEA4 (green) against a DAPI counterstain (blue).

Seamless transition from research-use Essential 8 Medium

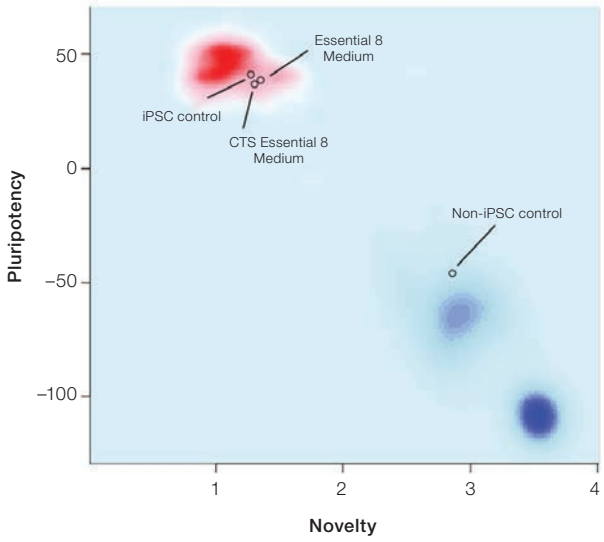


Figure 9. PluriTest™ tool for pluripotency analysis. PSCs were cultured in Essential 8 or CTS Essential 8 Medium for 5 passages and analyzed using the Applied Biosystems™ PrimeView™ 16 Global Gene Expression Profile Assay. Global gene expression was compared against pluripotent (red) and nonpluripotent (blue) reference data sets. Both samples clustered closely with the pluripotent control and reference set, and away from the nonpluripotent control and reference set.

Differentiation potential

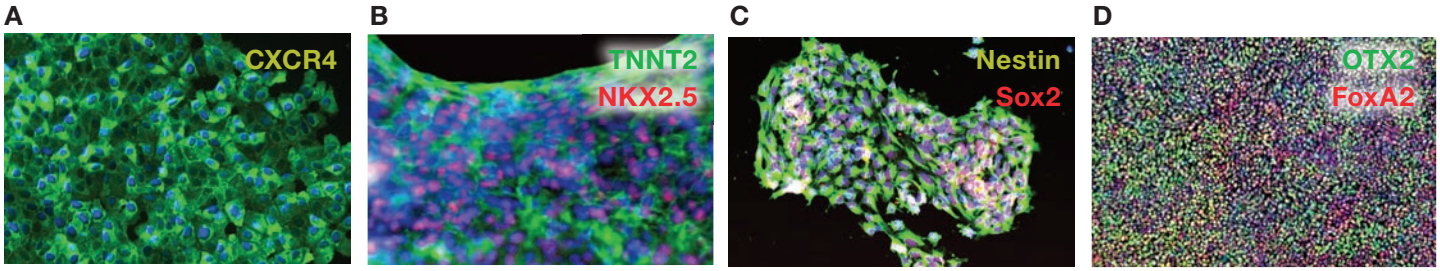


Figure 10. Tri-lineage differentiation via directed differentiation. PCSs were cultured in CTS Essential 8 Medium, and differentiation was induced using the following lineage-specific differentiation kits: **(A)** Gibco™ PSC Definitive Endoderm Induction Kit, **(B)** Gibco™ PSC Cardiomyocyte Differentiation Kit, **(C)** Gibco™ PSC Neural Induction Medium, and **(D)** Gibco™ Dopaminergic Neuron Differentiation Kit. The proper lineages are shown by **(A)** CXCR4 staining for definitive endoderm differentiation, **(B)** TNNT2 and NKX2.5 staining using the Invitrogen™ Human Cardiomyocyte Immunocytochemistry Kit for cardiomyocyte differentiation, **(C)** nestin and Sox2 staining using the Invitrogen™ Human Neural Stem Cell Immunocytochemistry Kit for neural stem cell differentiation, and **(D)** OTX2 and FoxA2 staining using the Invitrogen™ Human Dopaminergic Neuron Immunocytochemistry Kit for midbrain floor plate differentiation.

Differentiate PSCs into various lineages—Essential 6 Medium

Unlike other media used in PSC culture, Essential 6 Medium does not contain bFGF or TGFβ. Therefore, Essential 6 Medium can be used as a base for the differentiation of various cell types in the endodermal, mesodermal, and ectodermal lineages (Figures 11–13).

Neural stem cell differentiation

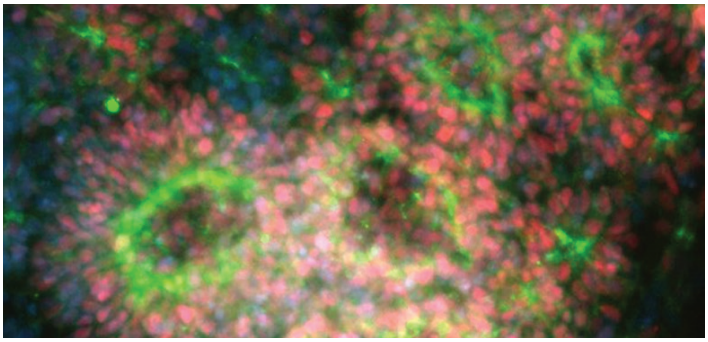


Figure 11. Neural stem cell (NSC) differentiation using Essential 6 Medium. PSCs cultured in Essential 8 Medium on rhVTN-N were differentiated into NSCs using Essential 6 Medium as a base, in accordance to Lippmann ES, et al. *Stem Cells* (2014). NSCs were stained with antibodies against Pax6 (red) and N-cadherin (green) with a DAPI counterstain (blue). (Data generated by Randolph Ashton, PhD and Nisha Iyer, PhD, University of Wisconsin, Madison)

Cardiomyocyte differentiation

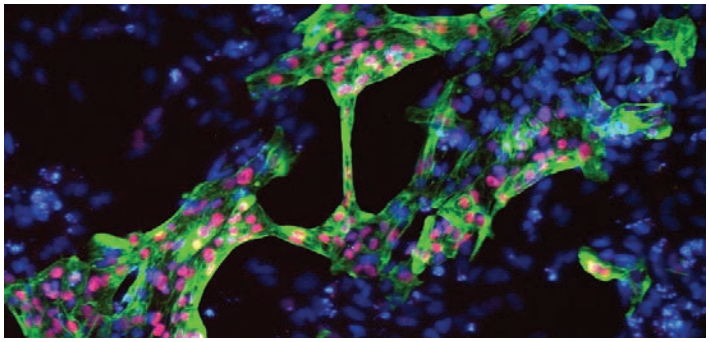
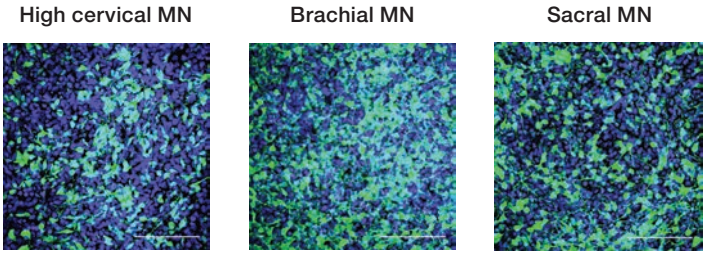


Figure 12. Cardiomyocyte differentiation using Essential 6 Medium. PSCs were differentiated into cardiomyocytes using WNT and GSK3B inhibitors in Essential 6 Medium. Cardiomyocytes were stained with antibodies for TNNT2 (green) and NKX2.5 (red) with a DAPI counterstain (blue).

Motor neuron differentiation

A



B

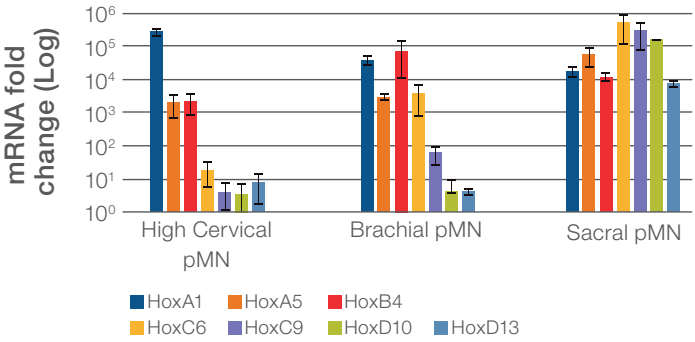


Figure 13. Motor neuron (MN) differentiation using Essential 6 Medium. Combinations of growth factors and small molecules were added sequentially to PSCs in Essential 6 Medium according to the protocol in Lippmann ES, et al. *Stem Cell Reports* (2015) in order to generate various types of motor neurons. **(A)** Expression of GFP (green) under the motor neuron-specific Hb9 promoter in the HUES3-HB9::GFP hESC line indicates differentiation into the motor neuron lineage. **(B)** RT-PCR analysis was performed and data were normalized at time 0 of CHIR treatment. The data reveal different Hox expression profiles indicative of regionalized motor neuron progenitors (pMN). (Data generated by Randolph Ashton, PhD and Nisha Iyer, PhD, University of Wisconsin, Madison)

Compatible with multiple PSC workflow reagents

Media system reagents, including matrix and passaging reagents, can have significant impact on downstream applications. Essential 8 and Essential 8 Flex media offer flexibility in the choice of reagents used within your culture system. Achieve optimal control regardless of your application, including expansion, single cell passaging, reprogramming, and differentiation (Table 3).

Table 3. Selection of PSC workflow reagents compatible with Essential 8 and Essential 8 Flex media.

Matrices	Passaging reagents	Cell banking	Reprogramming
Recombinant human vitronectin, truncated (rhVTN-N) Gibco™ Geltrex™ matrix (comparable to Matrigel™ matrix) Recombinant human Laminin-521	**Clump passaging** • EDTA or Gibco™ Versene™ Solution **Single cell passaging** • Gibco™ TrypLE™ Select Enzyme • Gibco™ RevitaCell™ Supplement • Gibco™ CTS™ TrypLE™ Select Enzyme	Gibco™ PSC Cryopreservation Kit RevitaCell Supplement	Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit

Transition from feeder-based to feeder-free culture conditions—Essential 8 Adaptation Kit

The Gibco™ Essential 8™ Adaptation Kit includes Essential 8 Medium and recombinant human Laminin-521 for optimal survival during the transition from a feeder-dependent to a feeder-free culture system.

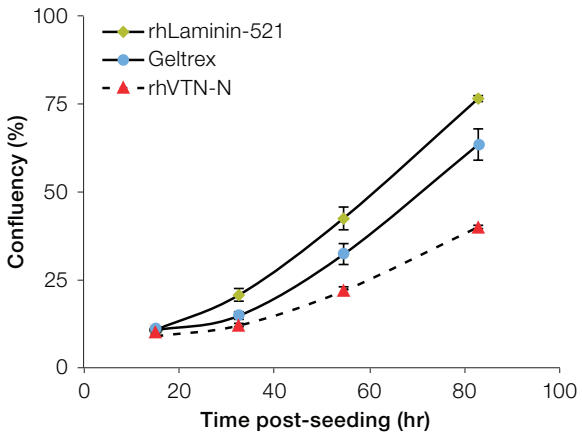


Figure 14. Optimum recovery of PSCs during transition to Essential 8 Medium in the absence of small molecule inhibitors. An episomal CD34+–derived iPSC line was cultured on mouse irradiated feeders in KSR-based medium prior to transition to Essential 8 Medium. For transition, colonies were passaged using collagenase IV per the protocol provided with the Essential 8 Adaptation Kit (Cat. No. A25935) and seeded into Essential 8 Medium in wells coated with rhLaminin-521 (green diamonds; A29248), Geltrex matrix (blue circles; Cat. No. A1413301), or recombinant human vitronectin (rhVTN-N) (red triangles; Cat. No. A14700). Confluency of cultures was monitored using the IncuCyte™ ZOOM system post-passage (n = 3 per condition).

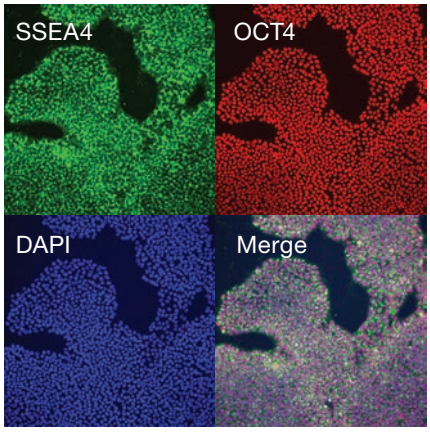


Figure 15. Human ESCs maintain expression of self-renewal factors. Human embryonic stem cells (ESCs) were grown under feeder-free conditions using Essential 8 Medium for 20 passages in wells coated with rhLaminin-521 (Cat. No. A29248). The cells were stained for pluripotency markers using the Invitrogen™ PSC 4-Marker ICC Kit (Cat. No. A24881).

Nurture cells through stressful events such as single cell passaging—Essential 8 Medium + RevitaCell Supplement

With the combination of Essential 8 Medium and RevitaCell Supplement, you can nurture your cells through difficult, stressful transitions, including single cell passaging and gene editing. For further support, combine these with a rhLaminin-521 substrate.

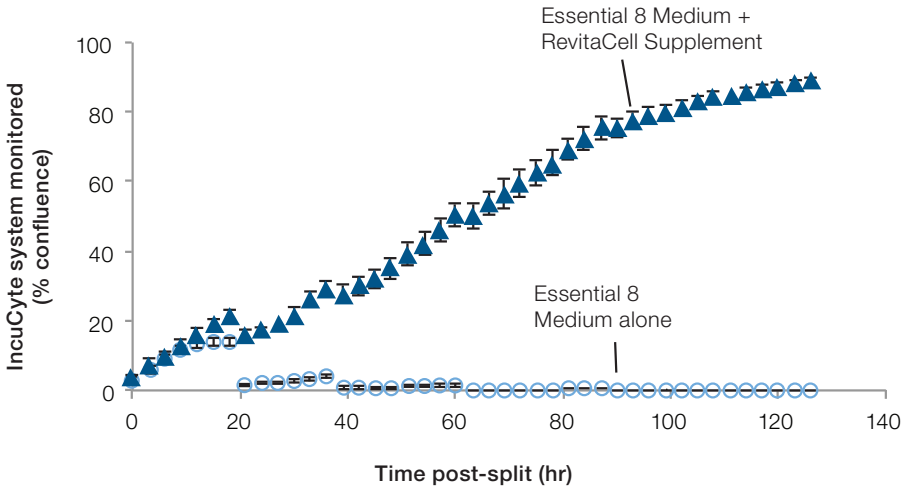


Figure 16. Single cell passaging using RevitaCell Supplement with Essential 8 Medium. iPSCs generated using episomal vectors were passaged using TrypLE Select Enzyme and seeded at a density of 25,000 viable cells/cm^2 onto culture plates coated with truncated recombinant human rhVTN, in Essential 8 Medium with 1X RevitaCell Supplement. Following a 24-hour recovery, iPSCs were fed with Essential 8 Medium alone for the remainder of their time in culture.

Support PSCs during reprogramming—Essential 8 or Essential 8 Flex Medium + CytoTune-iPS 2.0 Sendai Reprogramming Kit

Essential 8 media do not contain BSA, which has been shown to inhibit reprogramming in other feeder-free media formulations. Obtain high reprogramming efficiency while maintaining a defined culture environment with Essential 8 or Essential 8 Flex Medium. For translational research, we offer the CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit and CTS Essential 8 Medium.

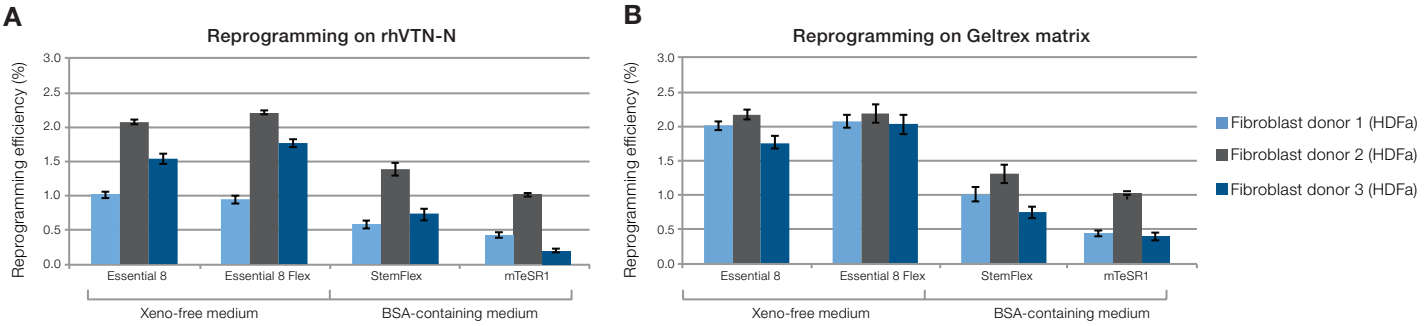
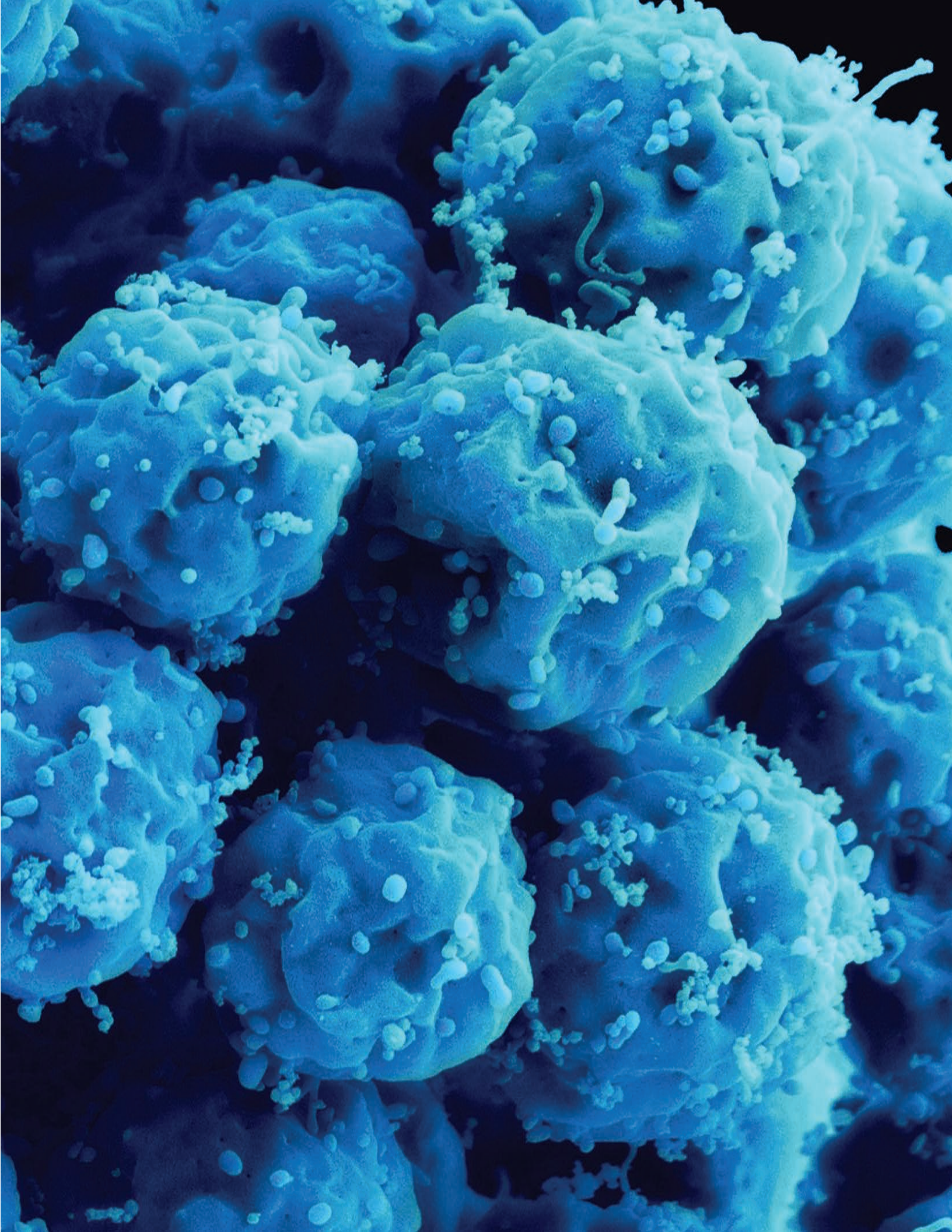


Figure 17. Reprogramming efficiency of human dermal fibroblasts using feeder-free medium conditions on Geltrex and rhVTN-N substrates. Fibroblasts from three donors—two adult and one neonatal—were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 7, 50,000 viable cells were transferred per well of a 6-well plate onto either **(A)** rhVTN-N or **(B)** Geltrex matrices, and on day 8 onward, were either fed daily with Essential 8 Medium or mTeSR1 Medium, or every other day with Essential 8 Flex Medium or Gibco™ StemFlex Medium. On day 21, alkaline phosphatase staining was completed and colony counting was performed using the IncuCyte™ ZOOM System to determine the reprogramming efficiency (percentage reprogramming efficiency = colonies counted/50,000 viable cells seeded x 100; n = 3 per condition).



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Ordering information

Application	Product	Cat. No.
Media	CTS Essential 8 Medium	A2656101
	Essential 6 Medium	A1516401
	Essential 8 Adaptation Kit	A25935
	Essential 8 Flex Medium	A2858501
	Essential 8 Medium	A1517001
	RevitaCell Supplement (100X)	A2644501
Reprogramming	CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit	A34546
	CytoTune-iPS 2.0 Sendai Reprogramming Kit	A16517
Matrices	Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A1413301
	rhLaminin-521	A29248
	Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Passaging reagents	CTS TrypLE Select Enzyme	A1285901
	RevitaCell Supplement (100X)	A2644501
	StemPro Accutase Cell Dissociation Reagent	A1110501
	TrypLE Select Enzyme (1X), phenol red	12563029
	Versene Solution	15040066
Cell banking	PSC Cryopreservation Kit	A2644601
	RevitaCell Supplement (100X)	A2644501

Essential 8 resources to support your success

Find the resources needed to support your PSC culture system when using Essential 8 media—no matter the application.

thermofisher.com/e8resources

Cellular Dynamics international

Take control today and go to
thermofisher.com/essentialmedia

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