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

ThermoFisher SCIENTIFIC

Today's stem cell challenges

Traditional media formulations for the culture of pluripotent stem cells (PSCs) were originally designed over a decade ago when the most important questions researchers were asking were about how can they reproducibly derive and maintain these cells. Over the course of the last ten years, the use of PSCs has expanded exponentially to more complex and intricate applications, including single-cell passaging and gene editing. Using the original culture media, researchers have had difficulty keeping up with the technological advances of the field when these media do not support the desired applications. Until now, there have been many protocol recommendations for traditional PSC media; however, these still leave significant performance gaps in next-generation applications of PSCs such as genome editing. In addition, these various protocol adjustments have created a new problem—inconsistent performance between researchers—making it difficult to reproduce results within and across labs.

Superior performance in modern applications

Over the past decade, the stem cell research community has been branching out beyond conventional approaches to more innovative technologies and applications, including genome editing and single-cell analysis. While these novel applications enable significant advancements in relevant physiological disease models, they put significant stress on stem cells, often impacting cell survival. Gibco™ StemFlex™ Medium is uniquely formulated to support cells through these stressful transitions while delivering consistent results and superior performance when compared to traditional media (Figures 1–3).

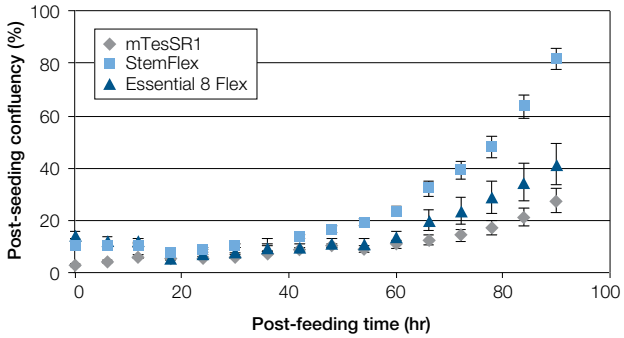


Figure 1. StemFlex Medium supports up to 2-fold faster recovery following gene editing. PSCs expanded in various media formulations were singularized using Gibco™ TrypLE™ Select enzyme and subjected to delivery of a Cas9 protein/HPRT guide RNA (gRNA) complex via electroporation. Upon seeding at 100,000 viable cells per well in the absence of Rho-associated protein kinase (ROCK) inhibitor, it was shown that StemFlex Medium supported optimal recovery of cells from this stressful event.

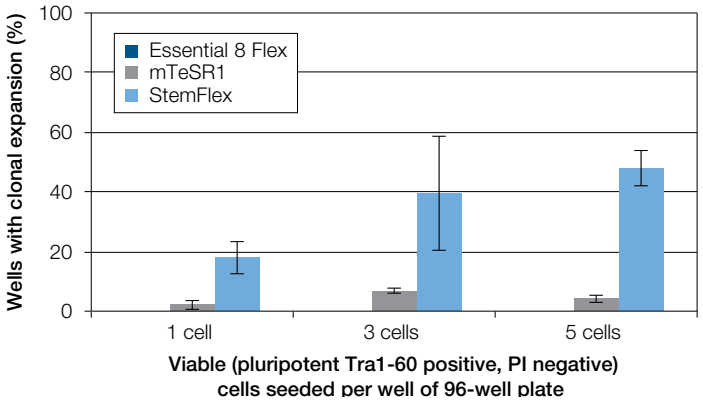
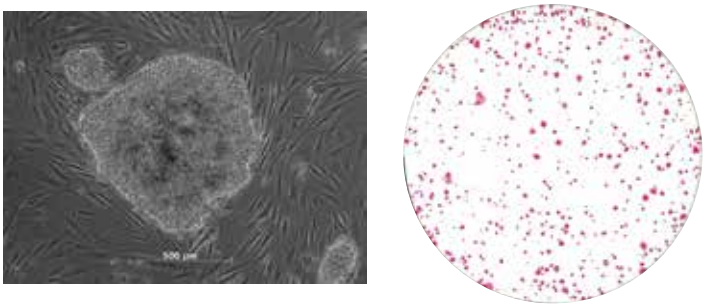


Figure 2. StemFlex Medium supports recovery of PSCs from flow sorting, demonstrating as much as 5-fold improvement in clonal expansion following single-cell passaging in the absence of ROCK inhibitor. PSCs expanded in StemFlex Medium on the rhLaminin-521 substrate for >3 passages were singularized using TrypLE Select enzyme, flow-sorted for live pluripotent stem cells (Tra1-60 positive, propidium iodide negative), and seeded at 1, 3, or 5 cells per well of a 96-well plate. Following plating, cells were fed with fresh medium every 3 days, and the percentage of wells attaining >5% confluency by day 14 was assessed via whole-well imaging on the IncuCyte™ ZOOM system (Essen Bioscience).

A



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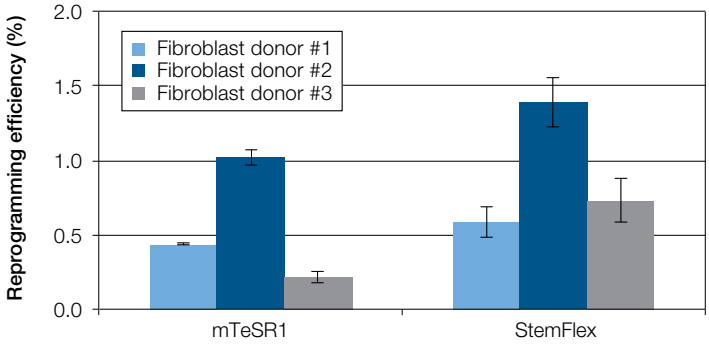


Figure 3. StemFlex Medium provides an improved workflow and robust colony formation for easy clone selection following reprogramming of somatic cells. Human dermal fibroblasts were reprogrammed using the Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Starting on day 8, when the stem cell medium is introduced per the standard workflow, cells were fed either daily with mTeSR™1 medium (STEMCELL Technologies) or every other day using the StemFlex Medium. **(A)** Robust colony formation was observed when StemFlex Medium was used. **(B)** Improved reprogramming efficiency was also observed for all three donors fed every other day using StemFlex Medium, relative to mTeSR1 cultures on a daily feed schedule.

Superior results with improved workflows

Traditionally, PSC culture has required daily feeding schedules to maintain pluripotency. This frequent handling of cells has created additional challenges for researchers, including the increased potential for errors and contamination, and increased variation should multiple users handle a single culture. As with the applications mentioned previously, protocol recommendations have been developed to ease some of these challenges with traditional culture systems. However, these recommendations are not well suited for continued, long-term use as they may have an impact on downstream pluripotency. StemFlex Medium (along with Gibco™ Essential 8™ Flex medium) enables a truly weekend-free feeding schedule (Figures 4 and 5) by consistently maintaining FGF-2 activity—a key factor in promoting pluripotency (Figure 6). Additionally, StemFlex Medium provides the flexibility to choose from various matrices and passaging reagents, depending on your experimental requirements (Table 1).

It is not uncommon for complementary reagents used within a PSC culture system (like matrices and passaging reagents) to have an unfavorable impact on performance. Traditional PSC media are typically only compatible with one matrix and one passaging method, which can require significant protocol adjustments or else may generate undesirable results. StemFlex Medium offers unprecedented three-way usage flexibility, where not only can you decide what matrix and passaging reagent will best fit your needs, but you can also choose your feeding schedule.

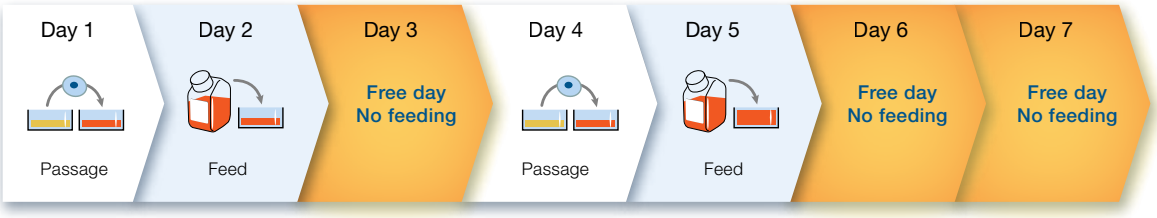


Figure 4. Recommended weekend-free feeding schedule. Unlike traditional PSC media, StemFlex Medium eliminates the need to manage cultures daily, enabling a truly weekend-free schedule for expansion and maintenance of PSCs. For additional feeding schedule options, visit **thermofisher.com/stemflex**

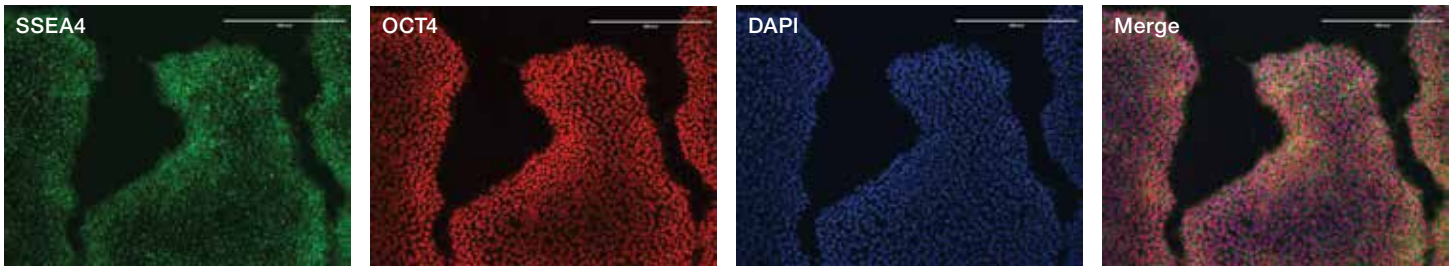


Figure 5. Long-term maintenance of pluripotency in weekend-free feeding. PSCs maintain expression of self-renewal factors when cultured in StemFlex Medium for 21 passages in wells coated with Gibco™ Geltrex™ matrix (Cat. No. A14133). The cells were stained for pluripotency markers using the Invitrogen™ PSC 4-Marker Immunocytochemistry Kit (Cat. No. A24881).

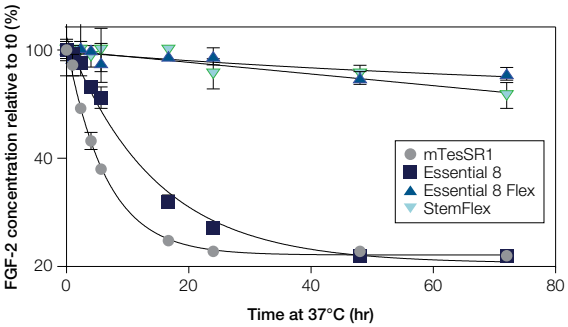


Figure 6. StemFlex Medium more consistently maintains pluripotency. StemFlex Medium provides prolonged FGF-2 stability when incubated at 37˚C, 5% CO_2, allowing for flexible feeding schedules, including the weekend-free option, and eliminating daily feeding requirements.

Table 1. Flexibility to choose the best matrix and passaging reagent.

Component	Product	Best for
Matrix	Gibco™ Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix	Replacement for Matrigel™ matrix; an ideal economical choice for expansion and maintenance of PSCs
	Vitronectin recombinant human protein, truncated (VTN-N)	Applications requiring leaner (xeno-free), defined matrix
	Recombinant human laminin-521 (rhLaminin-521)	Recommended for highest performance in stressful applications, including gene editing and single-cell passaging
Passaging reagent	Versene™ solution	Clump passaging
	Gibco™ StemPro™ Accutase™ reagent	2- to 3-cell clusters
	TrypLE Select Enzyme with or without RevitaCell™ Supplement	Single-cell passaging

A robust system for superior feeder-free culture of human PSCs

StemFlex Medium is our newest medium designed to deliver superior performance in the innovative and challenging applications used in today's stem cell research, such as cell reprogramming, single-cell passaging, and gene editing. In addition to core performance enhancements, it also delivers the convenience of a flexible feeding schedule (including weekend-free options) and the ability to choose between the matrix and passaging reagents best suited for a given application. StemFlex Medium is provided in a convenient, two-component kit (450 mL basal medium and 50 mL supplement), and when used with Geltrex matrix, provides a cost-effective, robust system for superior feeder-free culture of human PSCs.

As shown in Figures 7 and 8, StemFlex Medium enables long-term feeder-free culture of PSCs without karyotypic abnormalities for up to 21 passages with the weekend-free feeding schedule, and maintains the ability to differentiate the cells into all three germ layers.

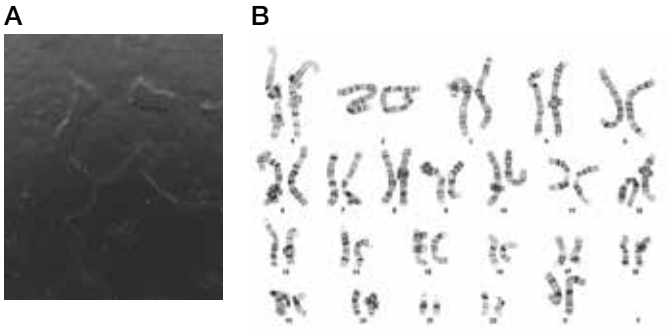


Figure 7. StemFlex Medium provides long-term maintenance of normal PSC properties. (A) PSC culture displays normal morphology. **(B)** A normal karyotype is observed following 20 passages on a weekend-free, flexible feeding schedule.

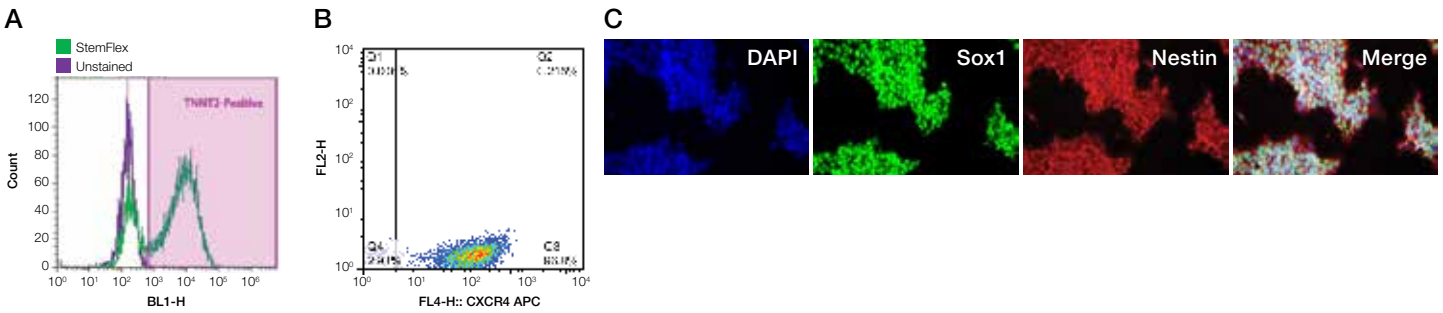


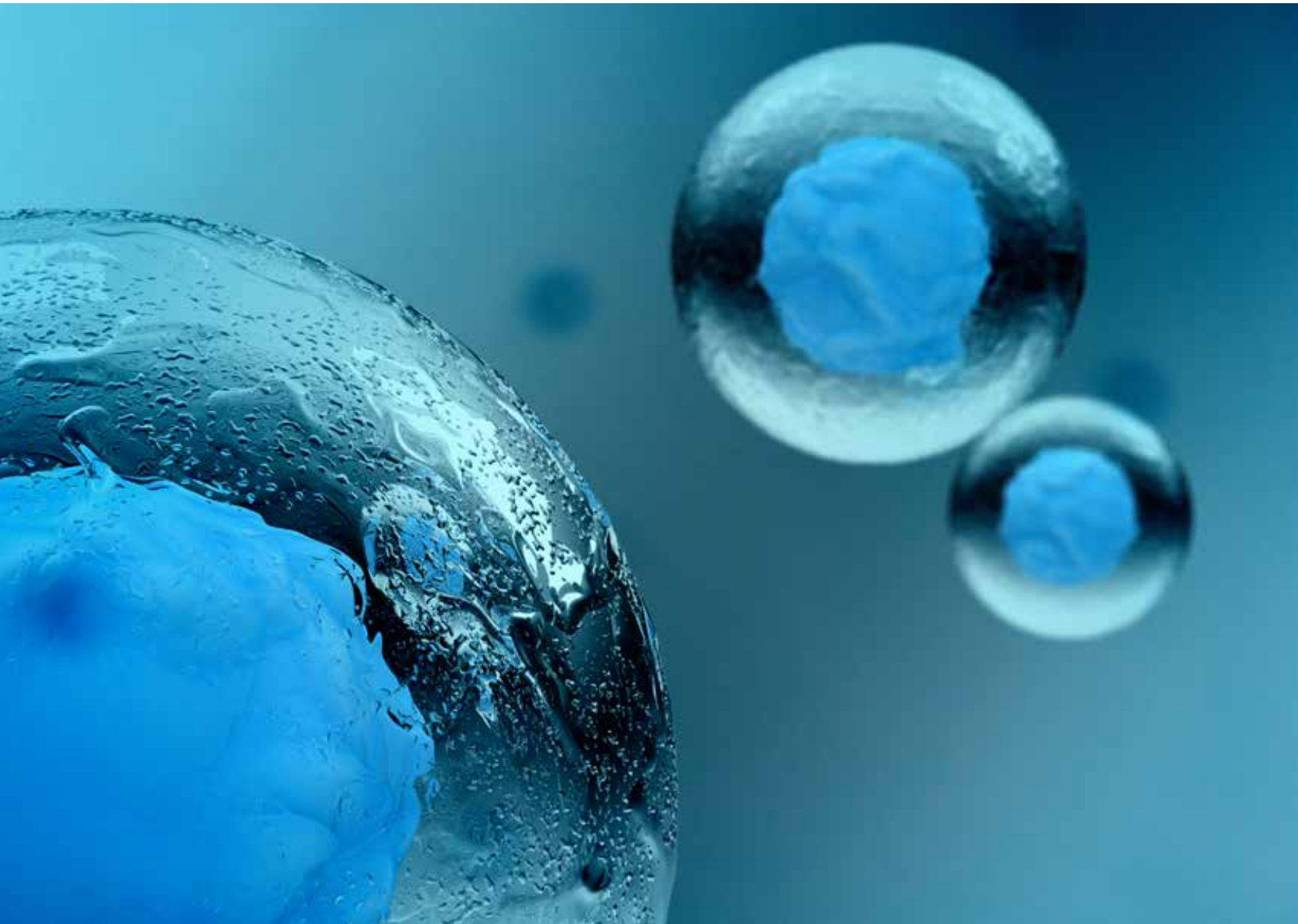
Figure 8. Confirmation of tri-lineage differentiation potential. Following at least 22 passages on the weekend-free feeding schedule, PSCs expanded in StemFlex Medium maintain the ability to differentiate into: **(A)** mesoderm, as shown by expression of TNNT2 following differentiation using the Gibco™ PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201), **(B)** endoderm, as shown by expression of the $CXCR4^+$, $PDGFRalpha^-$ phenotype, following differentiation using the Gibco™ PSC Definitive Endoderm Induction Kit (Cat. No. A3062601), and **(C)** ectoderm, as shown by expression of Sox1 and nestin following differentiation using the Gibco™ PSC Neural Induction Medium (Cat. No. A1647801).

Frequently asked questions (FAQs)

Question	Recommendation
Can the reconstituted StemFlex Medium be frozen for later use in usage-size aliquots?	Following reconstitution, the complete medium can be aliquoted and stored at –5°C to –20°C for up to 6 months. Alternatively, single-use aliquots of the supplement can be made and frozen at –5°C to –20°C. Avoid multiple freeze-thaw cycles.
How does StemFlex Medium compare to mTeSR1 and TeSR™-E8™ media?	StemFlex Medium is a more robust medium than TeSR-E8 medium and has fewer components than mTeSR1 medium. Performance of StemFlex Medium was shown to be superior to that of mTeSR1 medium in a number of applications, including single-cell passaging, gene editing, and reprogramming (Figures 3–5).
How difficult it is to adapt cells from another PSC medium, like mTeSR1 medium, to StemFlex Medium?	Adaptation of cells to StemFlex Medium from another PSC medium is simple and straightforward. For optimal results, we recommend a two-passage adaptation into StemFlex Medium. If cells are cryopreserved in mTeSR1 medium on Matrigel matrix, we recommend thawing cells back into the mTeSR1/Matrigel medium until fully recovered, then follow adaptation of cultures to StemFlex Medium using Versene solution for clump passaging.
If I am not interested in the weekend-free feeding schedule, can I still feed my cells daily?	StemFlex Medium will accommodate most feeding schedules, including every day and weekend-free. For a list of recommended feeding schedules, go to **thermofisher.com/stemflex**
For gene editing and/or flow sorting experiments, can antibiotics be used to prevent contamination?	Yes, antibiotics can be used within the StemFlex system to support gene editing and flow sorting. Gibco™ Antibiotic-Antimycotic (100X) (Cat. No. 15240096) is recommended.
What is the recommended protocol for optimal recovery during clonal expansion, for example, seeding of 1–5 cells per well of a 96 well-plate?	We recommend passaging cells on rhLaminin-521 ahead of flow sorting for at least 2 passages for optimal cell survival following single-cell passaging. Cells are then sorted onto the rhLaminin-521 matrix, and the recommended feed schedule for clonal recovery is every 3 days until day 14. NOTE: This feeding schedule is only recommended for low-density plating (i.e., 1–5 cells per well of a 96-well plate).
Is StemFlex Medium xeno-free and/or defined?	The formulation of StemFlex Medium includes bovine serum albumin (BSA) and thus is not considered xeno-free. It is more defined than mTeSR1 medium, as it contains fewer components in the formulation. Gibco™ Essential 8™ and Essential 8 Flex media are recommended as the most defined and xeno-free PSC culture media.

Ordering information

Product	Cat. No.
StemFlex Medium	A33494-01
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A14133
Vitronectin Recombinant Human Protein, Truncated (VTN-N)	A14700 and A31804
Recombinant Human Laminin-521 (rhLaminin-521)	A29248 and A29249
Versene Solution	15040
StemPro Accutase Cell Dissociation Reagent	A11105
TrypLE Select Enzyme	12563
RevitaCell Supplement	A2644501
PSC Cryopreservation Kit	A2644601



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

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