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Development of Feeder-Free PSC Culture System Enabling Translational & Clinical Research

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ABSTRACT

Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-origin-free alternatives for growth factors contained within the Essential 8™ Medium, producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with existing cGMP-manufactured passaging reagents: Versene Solution for clumped cell passaging and CTS™ TrypLE™ Select combined with RevitaCell™ Supplement for single cell passaging. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

INTRODUCTION

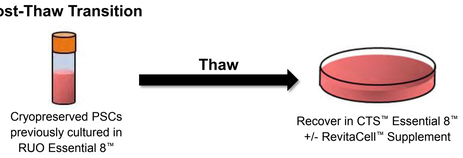
Figure 1. RUO to CTS™ Essential 8™ Media Conversion

	RUO Essential 8™ Medium	CTS™ Essential 8™ Medium
FDA Drug Master File (DMF)		✔
ISO & GMP manufacturing standards	✔	✔
Animal origin-free or xeno-free (primary component level)	Xeno-Free	Animal-Origin-Free
Certificates of Origin / Full Traceability		✔
Certificates of Analysis	✔	✔
H9 Performance Assay	✔	✔
Adventitious agent testing	✔	✔
USP Sterility testing	✔	✔
qPCR Mycoplasma testing		✔
Endotoxin testing	<12EU/mL	<1 EU/mL
Internal component regulatory risk assessment		✔
Intended use statement	For Research Use Only	For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products

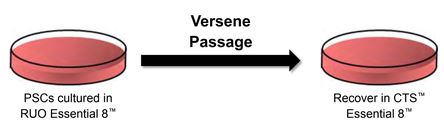
To provided a seamless transition from research to translational needs, the CTS™ Essential 8™ Medium is formulated with animal origin free growth factors and undergoes increased quality standards as highlighted in the table above.

Figure 2. Simple Transition options to transfer from RUO to CTS™ PSC Culture Systems

(A) Post-Thaw Transition



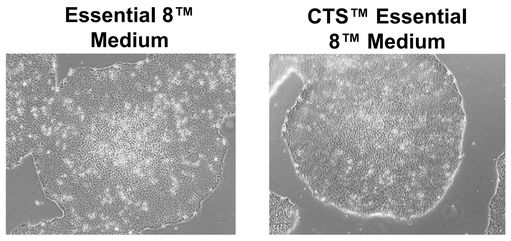
(B) Post-Passage Transition



Cultures previously cultured in the RUO PSC culture system can easily be transitioned to the CTS™ Essential 8™/ CTS™ rhVTN-N system using the above transition schemes.

RESULTS

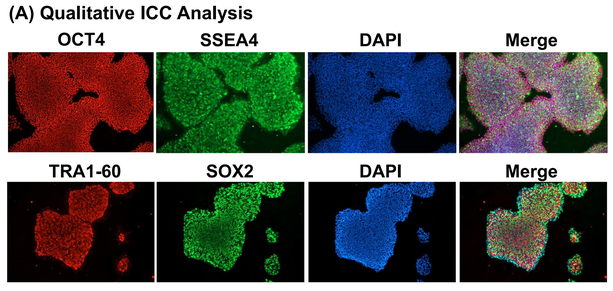
Figure 3. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N System Maintain Morphology Comparable to RUO System



Gibco™ Human Episomal iPSCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated for 10 passages using Versene Solution for passaging. PSCs were shown to maintain normal morphology as assessed by phase contrast imaging.

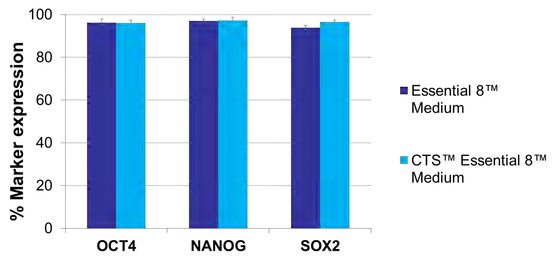
Figure 4. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N System Maintain Pluripotency

(A) Qualitative ICC Analysis

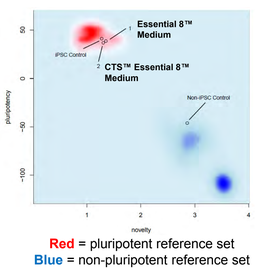


H9 ESCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated for >30 passages using Versene Solution for passaging. PSCs were shown to maintain normal pluripotency as assessed using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Cat. No. A24881).

(B) Quantitative ICC Analysis



(C) Pluritest™ Pluripotency Plot

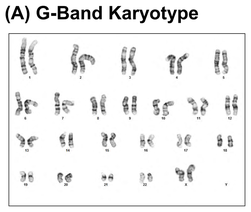


Red = pluripotent reference set
Blue = non-pluripotent reference set

Gibco™ Human Episomal iPSCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated for 3 passages using Versene Solution for passaging. PSCs were shown to maintain normal pluripotency as assessed by **(B)** Quantitative ICC using the Cellomics™ CellInsight™ and **(C)** Pluritest™, an open-access bioinformatic assay for pluripotency of human cells based upon their gene expression profiles.

Figure 5. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N System Maintain Normal Karyotype

(A) G-Band Karyotype



Gibco™ Human Episomal iPSCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated using Versene Solution for passaging. PSCs were shown to maintain normal karyotype as assessed (A) at P10 via G-Band Karyotype Assessment and (B) at P5 via Karyostat™ Assay .

(B) KaryoStat™ Assay

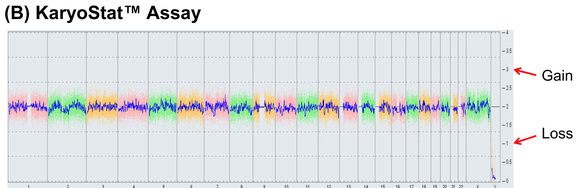
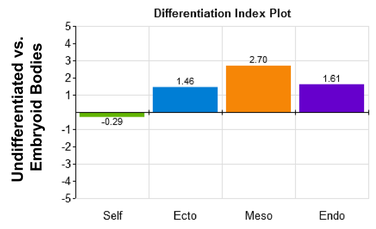


Figure 6. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N System Maintain Trilineage Differentiation Potential

(A) Trilineage Differentiation Potential Maintained As Assessed by TaqMan™ hPSC Scorecard™ Panel



(B) Trilineage Differentiation Potential Maintained As Assessed by Directed Differentiation Using Thermo Fisher Scientific Kits

Following expansion of PSCs in CTS™ Essential 8™ Medium on CTS™ rhVTN-N using Versene Solution for passaging, PSCs were evaluated for maintenance of trilineage differentiation potential via two methods. **(A)** Random differentiation of PSCs with subsequent evaluation using the TaqMan™ hPSC Scorecard™ Panel. (B) Directed differentiation using kits available from Thermo Fisher Scientific: differentiation to the endodermal lineage using the Gibco™ PSC Definitive Endoderm Induction Kit (Cat. No. A3062601), differentiation to mesodermal lineage using the Gibco™ PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201), and finally to the ectodermal lineage using the Gibco™ Neural Induction Medium (Cat. No. A1647801) and the Gibco™ Dopaminergic Neuron Differentiation Kit (Cat. No. A3147701).

Figure 7. Products Compatible with CTS™ Essential 8™ Medium

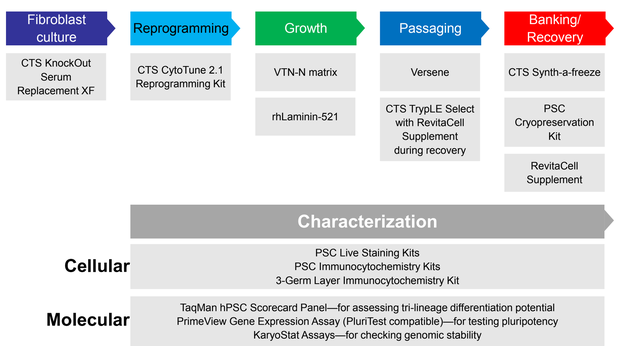
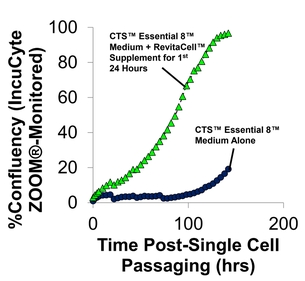


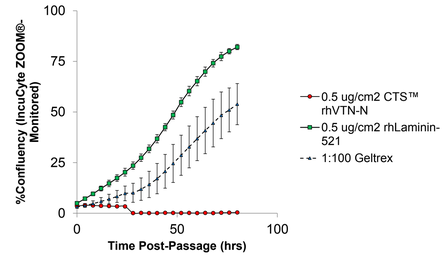
Figure 8. RevitaCell™ Supplement Provides Support When Single Cell Passaging is Required



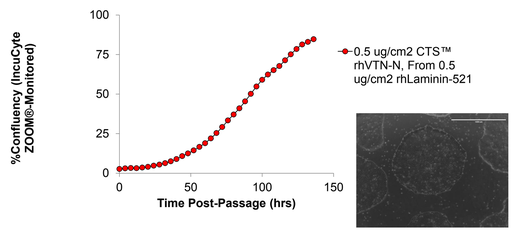
Gibco™ Human Episomal iPSC Line cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N was passaged using TrypLE™ Select and recovered at 25K viable cells/cm^2 in CTS™ Essential 8™ on CTS™ rhVTN-N (A) including (green triangles) or excluding (blue circles) RevitaCell™ Supplement (Cat. No. A26445-01) for the first 24 hours post-passage. Media was exchanged 24 hours post-passage with CTS™ Essential 8™ Medium alone.

Figure 9. rhLaminin-521 Can Be Used to Support Transition from Feeder-Dependent KSR-Based System to CTS™ Essential 8™/ CTS™ rhVTN-N System

(A) For Cell Lines Refractory to Transition, rhLaminin-521 Provides Optimum Cell Survival Post-Transition from KSR-Based Medium System to CTS™ Essential 8™



(B) PSCs Can Subsequently Be Transferred to CTS™ rhVTN-N for Remainder of Culture



(A) Feeder-Dependent iPSCs were collagenase passaged according to Essential 8™ Adaptation Kit protocol and seeded on various extracellular matrices for recovery in CTS™ Essential 8 Medium. rhLaminin-521 was shown to support optimum transition of challenging PSCs. (B) Cells are subsequently Versene passaged onto CTS™ rhVTN-N for the remainder of culture.

CONCLUSIONS

- The CTS™ Essential 8™/CTS™ rhVTN-N system provides a seamless transition from bench to clinic, providing long-term maintenance of normal PSC properties
- This CTS™ system provides reliable ancillary reagents for PSC culture upstream of manufacturing of cell, gene, or tissue-based products.
- The CTS™ system is compatible with existing reagents including RevitaCell™ Supplement for post-thaw recovery and single cell passaging applications, as well as compatible with Gibco™ differentiation kits.
- Please contact rhonda.newman@thermofisher.com or lauren.sangenario@thermofisher.com for additional information, including additional scale-up protocols using this medium system.

TRADEMARKS/LICENSING

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