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 and incorporate ISO13485 manufacturing for the recombinantly expressed, truncated human Vitronectin (rhVTN-N), 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

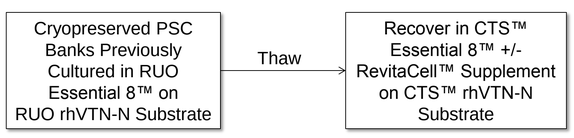
Figure 1. RUO to CTS™ Essential 8™ Media Conversion

	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

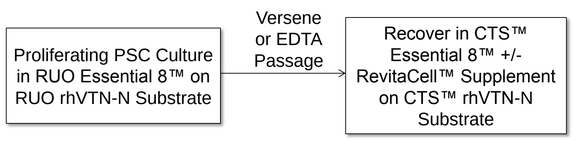
To provided a seamless transition from research to translational needs, the CTS™ Essential 8™ Medium is formulated with animal origin free growth factors and undergoes increased quality standards as highlighted in the table above.

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

(A) Post-Thaw Transition Scheme



(B) Post-Passage Transition Scheme

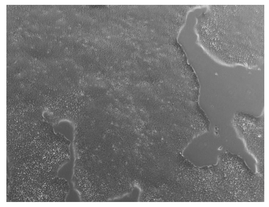


Cultures previously cultured in the RUO PSC culture system can easily be transitioned to the CTS™ Essential 8™/ CTS™ rhVTN-N system using the above transition schemes.

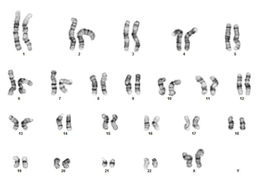
RESULTS

Figure 3. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N System Maintain Normal PSC Properties

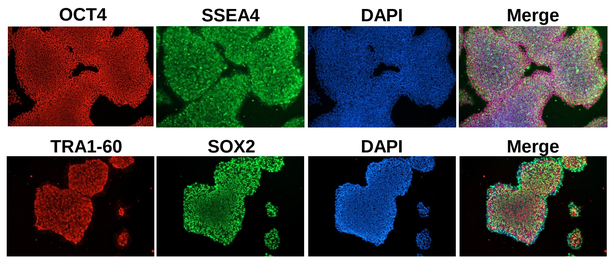
(A) Normal Morphology



(B) Normal Karyotype



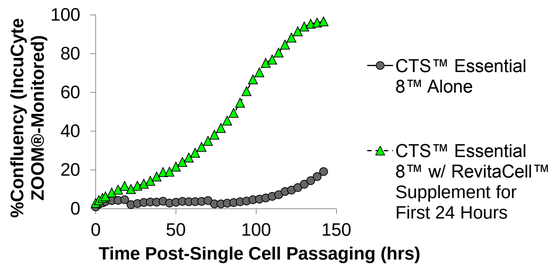
(C) Maintenance of Pluripotency



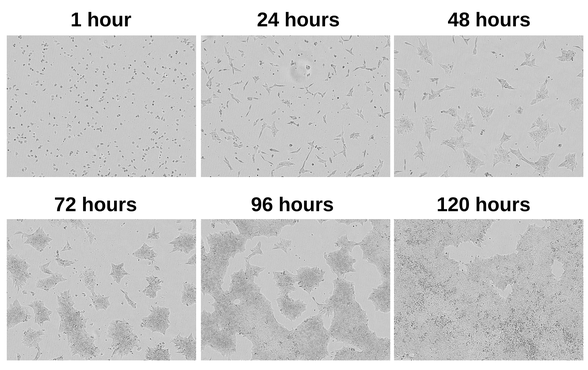
H9 ESCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated for >30 passages using Versene Solution for passaging. PSCs were shown to maintain normal (A) morphology as assessed by phase contrast microscopy using the EVOS® FL Cell Imaging Station, (B) karyotype as assessed by G-band Karyotyping, and (C) pluripotency as assessed using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Cat. No. A24881).

Figure 4. Use of RevitaCell™ Supplement Provides A Robust Single Cell Passaging Option for PSCs cultured in CTS™ Essential 8™/CTS™rhVTN-N System

(A) Recovery from Single Cell Passaging +/- RevitaCell™



(B) Representative Phase Contrast Images Following Recovery with RevitaCell™ Supplement

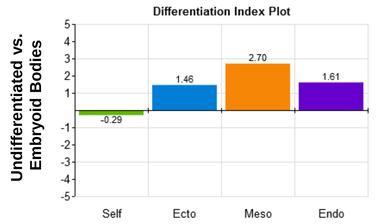


Gibco® Human Episomal iPSC Line cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N was single cell passaged using TrypLE™ Select Enzyme and recovered at 25,000 viable cells/cm^2 in CTS™ Essential 8™ on CTS™ rhVTN-N (A) including (green triangles) or excluding (blue circles) RevitaCell™ Supplement (Cat. No. A26445-01) for the first 24 hours post-passage. Media was exchanged 24 hours post-passage with CTS™ Essential 8™ Medium alone. (B) Representative images show characteristic morphology post single cell passaging over the time course monitored.

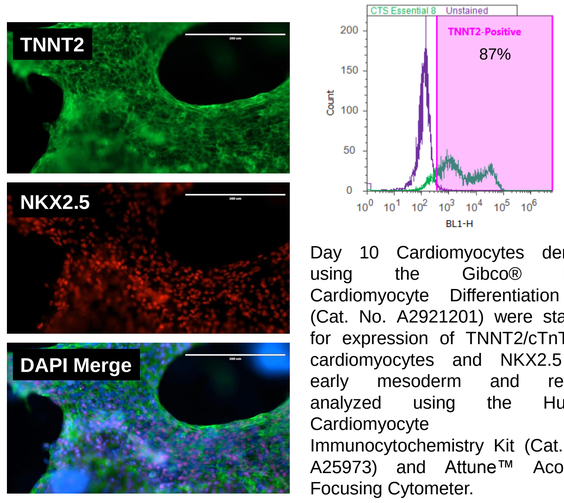
Addition of RevitaCell™ Supplement improves the robustness of this media system for downstream high throughput screening or differentiation experiments in which cells seeding consistency is required.

Figure 5. PSCs Cultured in CTS™ Essential 8™/ CTS™ rhVTN-N Maintain Trilineage Differentiation Potential

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

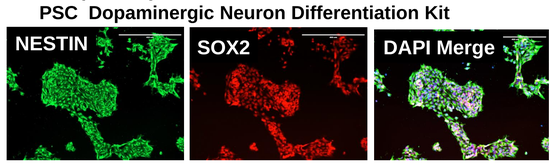


(B) Mesoderm Differentiation: Compatibility w/ Gibco® PSC Cardiomyocyte Differentiation Kit

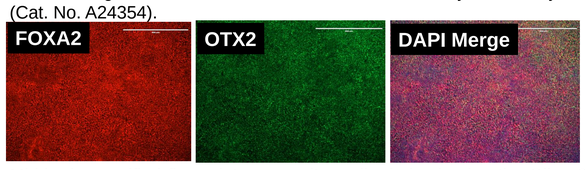


Day 10 Cardiomyocytes derived using the Gibco® PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201) were stained for expression of TNNT2/cTnT for cardiomyocytes and NKX2.5 for early mesoderm and results analyzed using the Human Cardiomyocyte Immunocytochemistry Kit (Cat. No. A25973) and Attune™ Acoustic Focusing Cytometer.

(C) Ectoderm Differentiation: Compatibility w/ Gibco® PSC Neural Induction Medium & PSC Dopaminergic Neuron Differentiation Kit

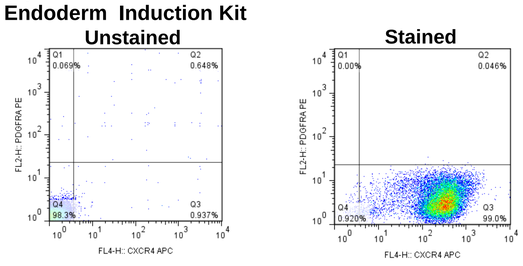


Neural stem cells derived using the Gibco® PSC Neural Induction Medium (Cat. No. A1647801) were stained for NSC markers, SOX2 and Nestin using the Human Neural Stem Cell Immunocytochemistry Kit (Cat. No. A24354).



Mid-brain specified floor plate progenitor cells derived using the Gibco® PSC Dopaminergic Neuron Differentiation Kit (Cat. No. A3147701) were stained for floor plate marker FOXA2 and rostral marker OTX2 using the Human Dopaminergic Neuron Immunocytochemistry Kit (Cat. No. A29515).

(D) Endoderm Differentiation: Gibco® PSC Definitive Endoderm Induction Kit

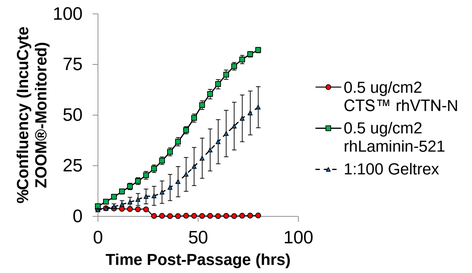


Definitive Endoderm cells derived using the Gibco® PSC Definitive Endoderm Induction Kit (Prototype; Cat. No. A27654SA) were stained for expression of CXCR4 and lack of expression of PDGFRα.

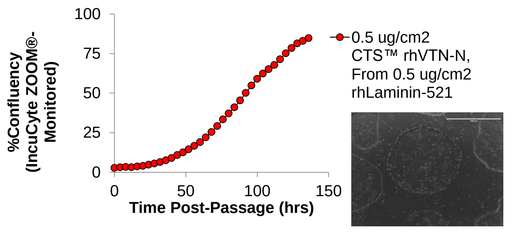
The CTS™ Essential 8™/CTS™ rhVTN-N System is shown to maintain trilineage differentiation potential of PSCs long-term and compatibility is shown with Gibco® differentiation kits. H9 ESCs cultured in CTS™ Essential 8™ Medium on CTS™ rhVTN-N were propagated for >30 passages using Versene Solution for passaging and subsequently assessed for maintenance of trilineage differentiation potential as shown above.

Figure 6. rhLaminin-521 Can Be Used to Support Transition from Feeder-Dependent KSR-Based System to CTS™ Essential 8™/ CTS™ rhVTN-N System

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



(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 long-term (>30 passages) maintenance of normal PSC properties, providing 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.
- The CTS™ Essential 8™ Medium and CTS™ rhVTN-N will be commercially available beginning in mid-September 2016. Please contact rhonda.newman@thermofisher.com or sandra.kuligowski@thermofisher.com for additional information regarding availability of these reagents.

TRADEMARKS/LICENSING