

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

Lauren E. Sangenar¹, Chad C. MacArthur², Uma Lakshmi², David T. Kuninger¹, & Rhonda A. Newman¹
Thermo Fisher Scientific, ¹Frederick, MD, ²Carlsbad, CA

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 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.

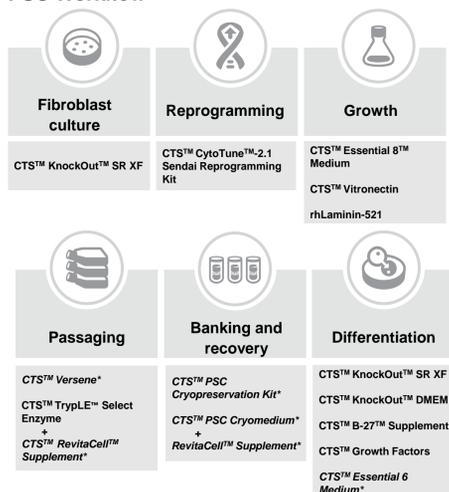
INTRODUCTION

Figure 1. How are Essential 8™ and CTS™ Essential 8™ Different?

	Essential 8™ Medium	CTS™ Essential 8™ Medium
ISO and GMP manufacturing standards	✓	✓
FDA Drug Master File (DMF)	N/A	✓
Animal origin-free (AOF) or xeno-free (XF)	XF	AOF
Full traceability	N/A	✓
H9 performance assay	✓	✓
Sterility testing	✓	✓
qPCR mycoplasma testing	N/A	✓
Endotoxin testing	<12 EU/mL	<1 EU/mL
Regulatory risk assessment	N/A	✓
Intended use statement	For Research Use Only	For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products

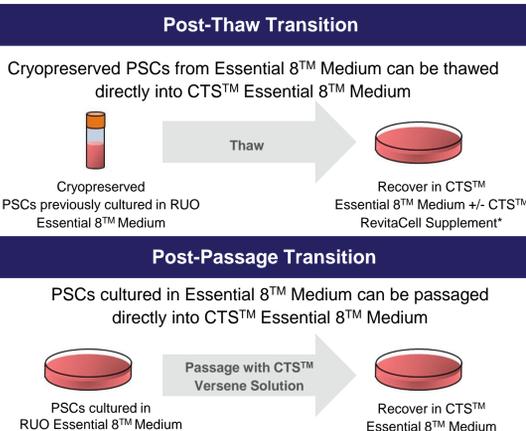
To provide a seamless transition from research to translational needs, the CTS™ Essential 8™ Medium is formulated with animal origin-free growth factors and undergoes increased quality standards as highlighted in the table above.

Figure 2. Cell Therapy Grade Products Available across the PSC Workflow



*These CTS Products Coming Soon

Figure 3. Simple Transition options to transfer from RUO to CTS™ PSC Culture Systems



RESULTS

Figure 4. PSCs Cultured in CTS™ Essential 8™/ rhVTN-N Formulation Maintain Normal PSC Properties

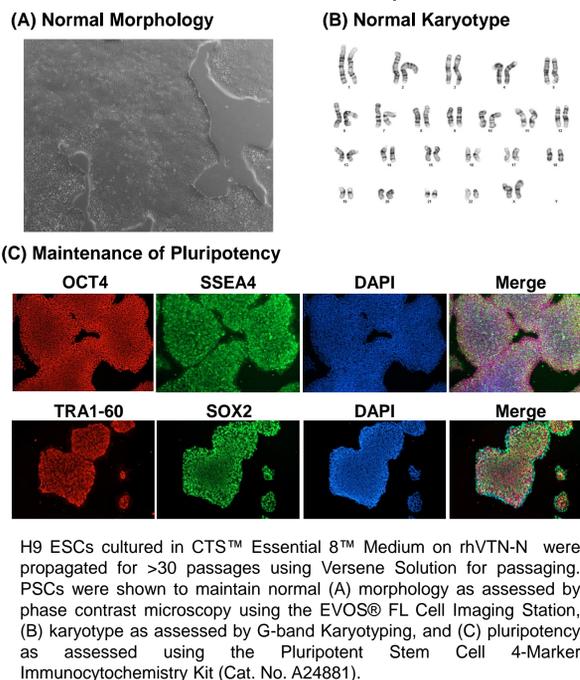
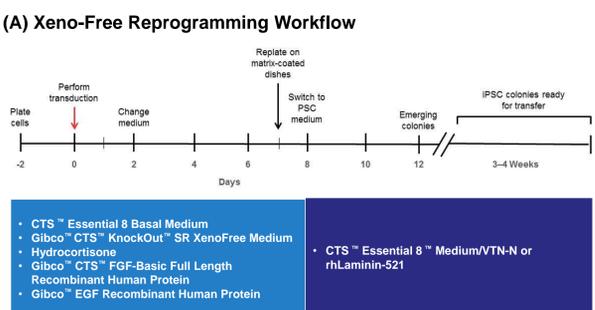
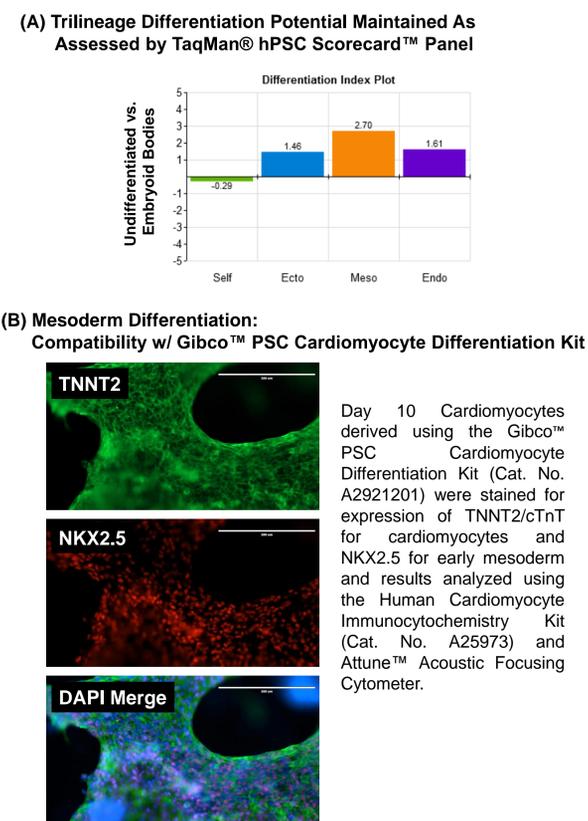


Figure 5. Compatibility with CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit for Reprogramming Fibroblasts in a Completely Xeno-Free Workflow

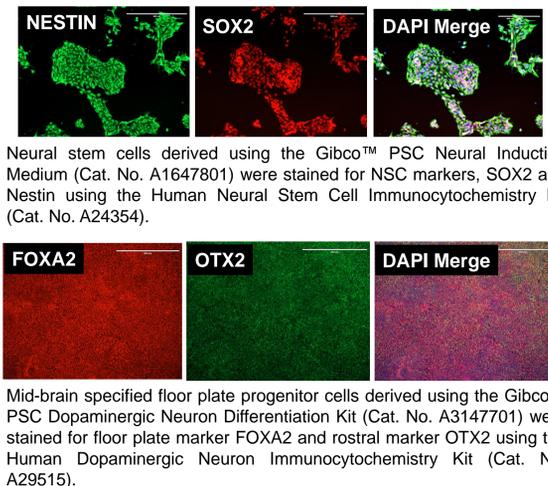


(A) Adult fibroblasts were plated then transduced with CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit in a xeno-free medium on a collagen matrix. At day 7, cells were harvested and replated on rhLaminin-521. The day after, medium was switched to research use only or CTS™ Essential 8™. (B) Reprogramming efficiency was determined 18 days post transduction by staining emerging colonies with alkaline phosphatase. Reprogramming efficiency was comparable for RUO and CTS™ Essential 8™ Medium.

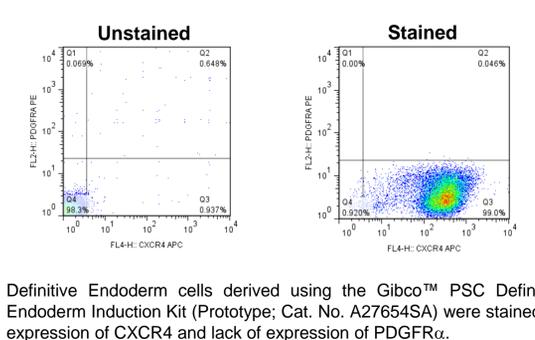
Figure 6. PSCs Cultured in the CTS™ Essential 8™/ rhVTN-N Formulation Maintain Trilineage Differentiation Potential



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

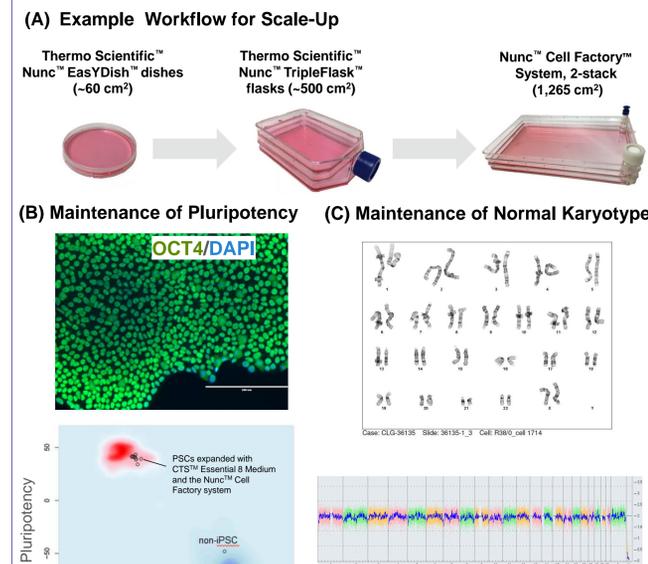


(D) Endoderm Differentiation: Gibco™ PSC Definitive Endoderm Induction Kit



PSCs cultured long-term in the CTS™ Essential 8™/rhVTN-N formulation are shown to maintain trilineage differentiation potential and compatibility with Gibco™ differentiation kits. H9 ESCs cultured in CTS™ Essential 8™ Medium on 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 7. Scalable PSC Expansion Coupling the CTS™ Essential 8™/ rhVTN-N System with Nunc™ Cell Factory™ System



(A) Gibco™ Episomal iPSCs were expanded in CTS™ Essential 8™ up to 350 million cells per 2-stack Nunc™ Cell Factory System™. CTS™ TrypLE™ Select, CTS™ RevitaCell™ Supplement* and CTS™ rhVTN-N are recommended for this workflow. (B) Post expansion, PSCs maintained pluripotency as assessed by OCT4 immunocytochemistry and the PluriTest Assay. (C) PSCs also maintained normal karyotype as assessed by G-banding & Karyostat Assays.

CONCLUSIONS

- The CTS™ Essential 8™/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 CTS™ RevitaCell™ Supplement* for post-thaw recovery and single cell passaging applications, as well as the CTS™ CytoTune™ -iPS 2.1 Sendai Reprogramming Kit and Gibco™ differentiation kits.
- These following PSC CTS™ products are now also available:
 - CTS™ Versene: Enables Clump Cell Passaging
 - CTS™ Essential 6™ Medium: Provides a Base Medium for Downstream Differentiation
 - CTS™ RevitaCell™ Supplement: Provides Efficient Recovery Following Cryopreservation & Single Cell Passaging
 - CTS™ PSC Cryomedium: Provides Optimum Security of Cell Supply

TRADEMARKS/LICENSING

© 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Essential 8 is a trademark of Cellular Dynamics International, Inc. TaqMan is a trademark of Roche Molecular Systems. For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.