

CTS Essential 8 Medium

A step forward for PSC-based cell therapy

Gibco™ CTS™ Essential 8™ Medium is the first globally available culture medium for human pluripotent stem cells (PSCs) formulated with components not directly derived from animals. Based on Gibco™ Essential 8™ Medium, CTS Essential 8 Medium enables a seamless transition from the bench to the clinic.

Why use it for clinical applications?

- **Helps reduce risks**—fully defined
- **Facilitates regulatory filings**—manufactured in accordance with cGMP regulations for medical devices, 21 CFR Part 820 and ISO 13485, with access to regulatory documentation, including FDA Drug Master File
- **Seamless transition**—same 8-component formulation as our research-use Gibco™ Essential 8™ Medium, but with components not directly derived from animals (Table 1)



Table 1. Features of Essential 8 Medium vs. CTS Essential 8 Medium.

Feature	Essential 8 Medium	CTS Essential 8 Medium
FDA Drug Master File	N/A	•
Xeno-free (XF) or animal origin-free (AOF)	XF	AOF
Regulatory risk assessment	N/A	•
Full traceability	N/A	•
Endotoxin testing	<12 EU/mL	<1 EU/mL
qPCR mycoplasma testing	N/A	•
Adventitious agent testing	•	•
USP sterility testing	•	•
H9 performance assay	•	•
cGMP manufacturing	•	•
Intended use statement	For Research Use Only. Not for use in diagnostic procedures.	For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. Caution: Not intended for direct administration into humans or animals.

Long-term maintenance of PSC cultures

PSCs have been shown to maintain PSC marker expression and normal karyotype after long-term culture in the CTS Essential 8 formulation (Figures 1 and 2).

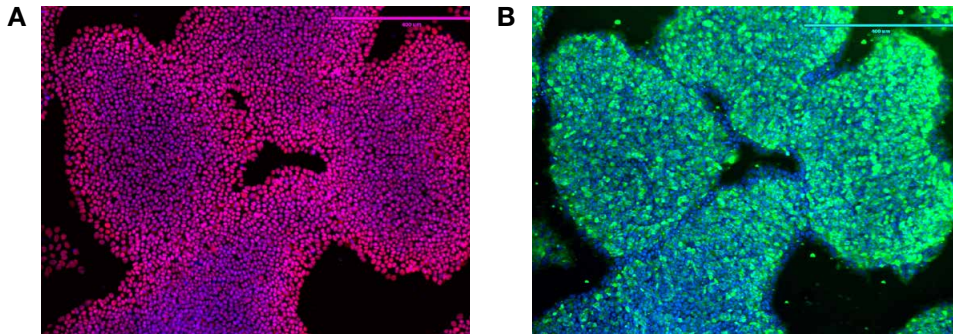


Figure 1. PSC marker expression. PSCs were cultured in CTS Essential 8 Medium for over 30 passages and then stained using the Invitrogen™ PSC 4-Marker Immunocytochemistry Kit. PSCs exhibit strong expression of the PSC markers (A) Oct4 (red) and (B) SSEA4 (green) against a DAPI counterstain (blue).

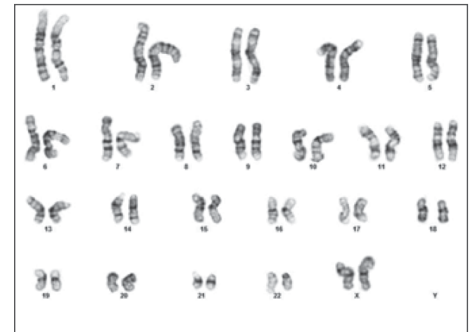


Figure 2. Normal karyotype. Gibco™ iPSCs cultured in CTS Essential 8 Medium for 10 passages were analyzed by G-banding to visualize chromosomes. The iPSCs were found to have a normal 46, XX karyotype.

Differentiation potential

PSCs cultured in the CTS Essential 8 formulation have been shown to generate cells of the three germ lineages (Figure 3).

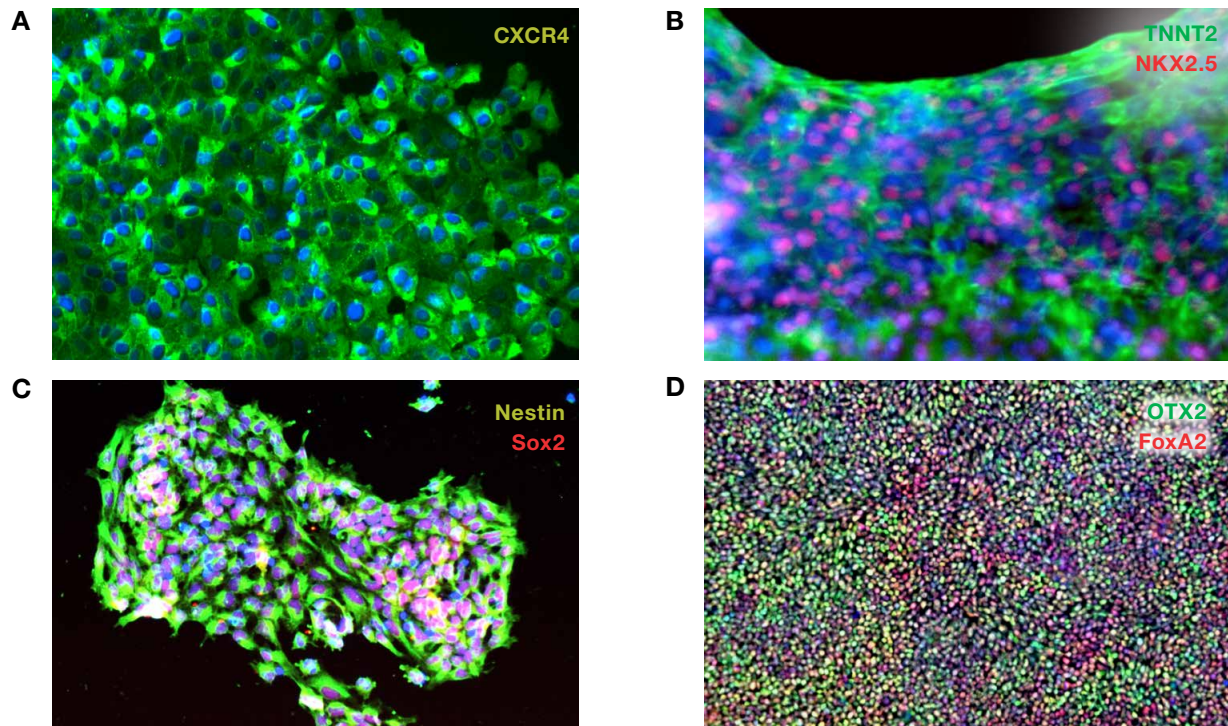


Figure 3. Trilineage differentiation via directed differentiation. PSCs were cultured in CTS Essential 8 Medium, and differentiation was induced using the following lineage-specific differentiation kits: (A) Gibco™ PSC Definitive Endoderm Induction Kit, (B) Gibco™ PSC Cardiomyocyte Differentiation Kit, (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.

Seamless transition from research-use Essential 8 Medium

PSCs cultured in CTS Essential 8 Medium were shown to be comparable to PSCs cultured in research-use Essential 8 Medium (Figures 4-6).

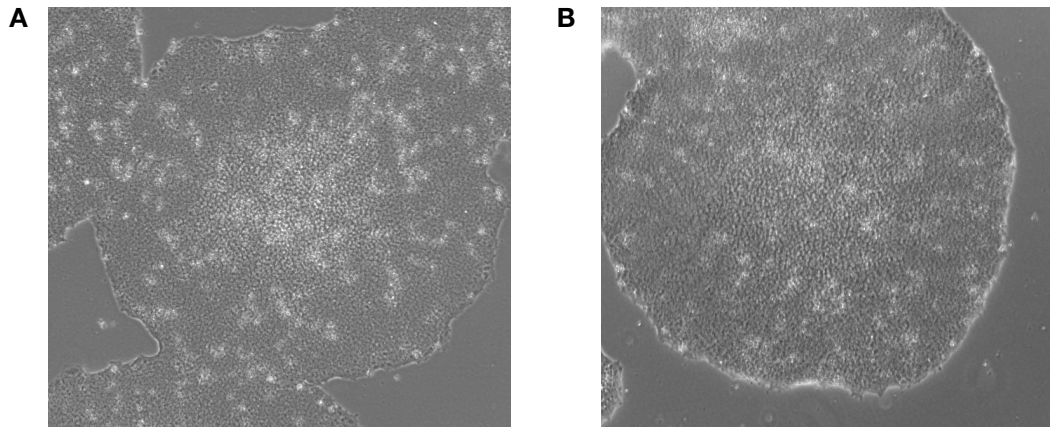


Figure 4. Comparison of PSC colony morphology. PSCs were cultured in (A) Essential 8 or (B) CTS Essential 8 Medium. At passage 10, PSCs were imaged using phase-contrast microscopy. PSCs cultured in CTS Essential 8 Medium form compact colonies with defined edges, similar to colonies cultured in research-use Essential 8 Medium.

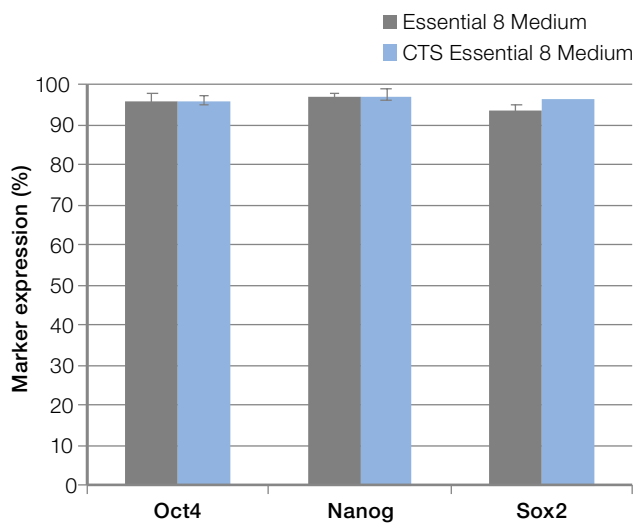


Figure 5. Comparison of PSC marker expression. PSCs were cultured in Essential 8 or CTS Essential 8 Medium. At passage 3, positive staining for PSC markers was quantified to obtain percent marker expression. High percentages of cells expressed PSC markers whether they were cultured in research-use Essential 8 Medium or CTS Essential 8 Medium.

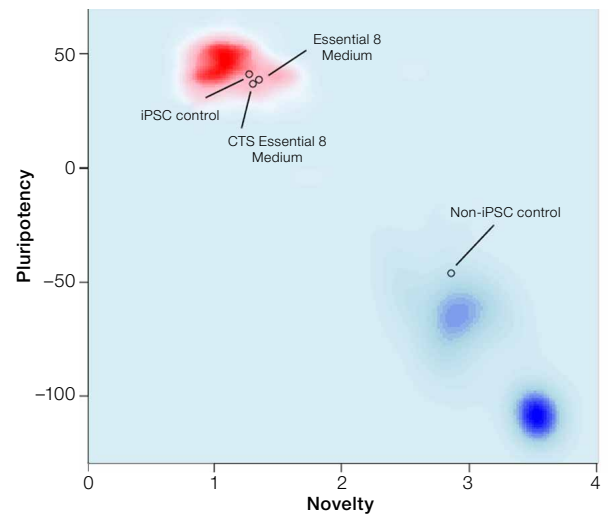


Figure 6. PluriTest™ tool for pluripotency analysis. PSCs were cultured in Essential 8 or CTS Essential 8 Medium for 5 passages and analyzed using the Applied Biosystems™ PrimeView™ 16 Global Gene Expression Profile Assay. Global gene expression was compared against pluripotent (red) and nonpluripotent (blue) reference data sets. Both samples clustered closely with the pluripotent control and reference set, and away from the nonpluripotent control and reference set.

Pluripotent stem cell therapy workflow solutions

We support the development of your iPSC therapy from the earliest stages of research all the way to the clinic. We offer high-quality Research Use Only products to support your early cell therapy development and CTS products as

you move toward clinical and commercial applications. Our GMP-manufactured CTS products offer specific cell and gene therapy intended use statements, extensive safety testing, and proactive regulatory documentation.



Reprogramming

Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit



Banking and recovery

Gibco™ CTS™ PSC Cryopreservation Kit
Gibco™ CTS™ PSC Cryomedium
Gibco™ CTS™ RevitaCell™ Supplement



Expansion and gene editing

Expansion media systems
CTS Essential 8 Medium
Gibco™ CTS™ Vitronectin (VTN-N)
Invitrogen™ Recombinant Human Protein
Gibco™ rhLaminin-521
Gibco™ CTS™ Versene™ Solution
Gibco™ CTS™ TrypLE™ Select Enzyme
CTS RevitaCell Supplement

Genome editing
Gibco™ TrueCut™ Cas9 Protein (CTS™-Prototype)
Invitrogen™ TrueGuide™ Synthetic gRNAs
Invitrogen™ Neon™ Transfection System



Differentiation

Gibco™ CTS™ Essential 6™ Medium
Gibco™ CTS™ KnockOut™ SR XenoFree Medium
Gibco™ CTS™ N-2 Supplement
Gibco™ B-27™ Supplement, XenoFree
Gibco™ CultureOne™ Supplement

Characterization

Applied Biosystems™ TaqMan® hPSC Scorecard Panel—for assessing trilineage differentiation potential
Applied Biosystems™ PrimeView™ gene expression assays (compatible with the PluriTest assay)—for testing pluripotency
Applied Biosystems™ KaryoStat™ Assays—for checking genomic stability
Invitrogen™ CellModel™ Services—for stem cell characterization

Ordering information

Product	Cat. No.
B-27 Supplement (50X), serum free	17504044
CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit	A34546
CTS Essential 6 Medium	A4238501
CTS Essential 8 Medium	A2656101
CTS KnockOut SR XenoFree Medium	12618012
CTS N-2 Supplement	A1370701
CTS PSC Cryopreservation Kit	A4239301
CTS PSC Cryomedium	A4238801
CTS RevitaCell Supplement (100X)	A4238401
CTS TrypLE Select Enzyme	A1285901
CTS Versene Solution	A4239101
CTS Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A27940
CultureOne Supplement (100X)	A3320201
Neon Transfection System	MPK5000
rhLaminin-521	A29248
TrueCut Cas9 Protein (CTS-Prototype)	A45220P

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