CTS™ Essential 8™ Medium

Catalog Number A2656101

Pub. No. MAN0010994 Rev. 3.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Gibco™ CTS™ Essential 8™ Medium (Cat. No. A2656101) is a fully defined feeder-free medium formulated for the growth and expansion of human pluripotent stem cells (PSCs) and contains no animal- or human-derived components at the primary level, enabling consistent PSC culture conditions for translational and clinical research.

CTS™ Essential 8™ Medium is manufactured at a site that uses methods and controls that conform with cGMP for medical devices, 21 CFR Part 820.

Contents and storage

Contents[1]	Cat. No.	Amount	Storage	Shelf life[2]
CTS™ Essential 8™ Medium Kit, (Cat. No. A2656101)				
CTS™ Essential 8™ Basal Medium	A2655901	500 mL	2°C to 8°C. Protect from light.	12 months
CTS™ Essential 8™ Supplement [3]	A2656001	10 mL	-20°C to -5°C. Protect from light.	

[1] CTS™ Essential 8™ Medium is sold as a complete kit; individual components are not sold separately.
[2] Shelf-Life duration is determined from Date of Manufacture.
[3] Store the CTS™ Essential 8™ Supplement in a non-frost-free freezer at -20°C to -5°C. Do not refreeze the thawed supplement.

Culture conditions

Media: Complete CTS™ Essential 8™ Medium

Culture type: Adherent

Recommended substrates: CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A27940), rhLaminin-521 (Cat. No. A29249)

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Procedural guidelines

- Thaw frozen CTS™ Essential 8™ Supplement at room temperature for ~1 hour to prepare complete medium
- Complete CTS™ Essential 8™ Medium can be stored at 2°C to 8°C for up to 2 weeks.
- Do not thaw the frozen supplement at 37°C
- Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch.

IMPORTANT! Do not warm the medium at 37°C.

Prepare complete CTS™ Essential 8™ Medium

1. Thaw the frozen CTS™ Essential 8™ Supplement at room temperature for ~1 hour.

   Do not thaw the frozen supplement at 37°C

2. Mix the thawed supplement by gently inverting the vial a couple of times.

3. Remove 10 mL from the bottle of CTS™ Essential 8™ Basal Medium, then aseptically transfer 10 mL of CTS™ Essential 8™ Supplement to the bottle of CTS™ Essential 8™ Basal Medium.

4. Swirl the bottle to obtain 500 mL of homogenous complete medium.

Guidelines to culture human PSCs in CTS™ Essential 8™ Medium

- Split cultures when the first of the following occurs:
  - PSC colonies are becoming too dense or too large
  - PSC colonies are showing increased differentiation
  - The colonies cover ~85% of the surface area of the culture vessel, usually every 4 to 5 days

- The split ratio can vary, though it is generally between 1:2 and 1:4 for early passages and between 1:3 and 1:12 for established cultures. Occasionally, cells will grow at a different rate and the split ratio will need to be adjusted.
- A general rule is to observe the last split ratio and adjust the ratio according to the appearance of the PSC colonies. If the cells look healthy and the colonies have enough space, split using the same ratio. If the colonies are overly dense and crowding, increase the ratio; if they are sparse, decrease the ratio.
- Newly derived PSC lines may contain a fair amount of differentiation through passage 4. It is not necessary to remove differentiated material prior to passaging. By propagating/splitting the cells, the overall culture health should improve throughout the early passages.
- Do not scrape the cells from the culture vessel during passaging.

Recover frozen PSCs in complete CTS™ Essential 8™ Medium

1. Pre-warm complete CTS™ Essential 8™ Medium and CTS™ VTN-N-coated 6-well plates to room temperature.

   See *CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated User Guide* (available at **thermofisher.com**).

2. Remove the vial of PSCs from liquid nitrogen storage and transfer it on dry ice to the tissue culture room.

3. Immerse the vial in a 37°C water bath without submerging the cap.

   Swirl the vial gently.

4. When only an ice crystal remains, remove the vial from the water bath, spray the outside with 70% ethanol, and place it in the hood.

5. Transfer the thawed cells to a 15-mL conical tube and slowly add 10 mL of complete CTS™ Essential 8™ Medium drop-wise to the cells.

   This reduces osmotic shock to the cells.

6. While adding the medium, gently move the tube back and forth to mix the PSCs.

7. Rinse the vial with 1 mL of complete CTS™ Essential 8™ Medium and add to the 15-mL tube with cells.

8. Centrifuge the cells at 200 × *g* for 5 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 2 mL of complete CTS™ Essential 8™ Medium by gently pipetting the cells up and down a few times.

9. Slowly add the PSC suspension into pre-warmed, CTS™ VTN-N-coated 6-well plate, plating ~1 million viable cells per well of a 6-well plate.

   Optional: To improve efficiency of cell survival 24 hours post-thaw, chemically defined, animal origin-free, CTS™ RevitaCell™ Supplement (Cat No. A4238401) may be used at 1X final concentration (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-thaw. If using CTS™ RevitaCell™ Supplement for recovery of your PSCs, lower cell seeding densities are required; plating at a viable cell density of ~40,000 viable cells/cm^2 will allow for recovery in 3–4 days post-thaw.

10. Move the plate in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the wells and place the plate gently into the 37°C, 5% CO_2 incubator.

11. The next day, replace the spent medium with fresh complete CTS™ Essential 8™ Medium.

    Replace the medium daily thereafter until the cells are approximately 85% confluent.

Passage PSCs with CTS™ Versene Solution

See “Recommended plating volumes” on page 3 for recommended volumes.

1. Pre-warm complete CTS™ Essential 8™ Medium, CTS™ VTN-N-coated culture vessels, and the CTS™ Versene Solution to room temperature.

2. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel with CTS™ DPBS without calcium chloride, without magnesium chloride.

3. Add CTS™ Versene Solution to the vessel containing PSCs. Swirl the vessel to coat the entire well surface.

4. Incubate the vessel at room temperature for 5 to 8 minutes or at 37°C for 4 to 5 minutes.

   When the cells start to separate and round up, and the colonies appear to have holes when viewed under a microscope, they are ready to be removed from the vessel.

5. Aspirate the CTS™ Versene Solution and add pre-warmed complete CTS™ Essential 8™ Medium to the vessel.

6. Remove the cells from the well(s) by gently squirting medium over the surface of the well 2–3 times and pipetting the colonies up with a 5 mL serological pipette.

   Do not pipet vigorously or the colonies will break apart. Avoid creating bubbles.

7. Collect cells in a 15-mL conical tube.

   There may be obvious patches of cells that were not dislodged. Do not scrape the cells from the dish in an attempt to recover them.

   Note: Depending upon the cell line, work with no more than 1 to 3 wells at a time, and work quickly to remove cells after adding CTS™ Essential 8™ Medium to the well(s), which quickly neutralizes the initial effect of the CTS™ Versene Solution. Some lines re-adhere very rapidly after medium

addition, and must be removed 1 well at a time. Others are slower to re-attach, and may be removed 3 wells at a time.

8. Add an appropriate volume of pre-warmed complete CTS™ Essential 8™ Medium to the CTS™ VTN-N-coated vessel so that each well contains the appropriate volume of complete medium after the cell suspension has been added.

9. Mix the cell suspensions from step 7 by gentle inversion a few times and transfer the appropriate volume of cell suspension into each well containing pre-warmed complete CTS™ Essential 8™ Medium.

10. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the wells.

11. Incubate the cells in the 37°C, 5% CO_2 incubator overnight.

12. Feed the PSCs on the day after splitting. Replace the spent medium daily.

    Note: It is normal to see cell debris and small colonies after passage.

    Optional: To improve efficiency of cell survival 24 hours post passaging, chemically defined, animal-origin free CTS™ RevitaCell™ Supplement (Cat. No. A4238401) may be used at 1X final concentration (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-passage to minimize apoptosis and necrosis.

Recommended plating volumes

Table 1 Reagent volumes in (mL per well or per dish)

Culture vessel (approx. surface area)	1X CTS™ VTN-N solution[1]	CTS™ DPBS	CTS™ Versene Solution	Complete medium
6-well (10 cm^2)	1 mL	2 mL	1 mL	2 mL
12-well (4 cm^2)	0.4 mL	1 mL	0.4 mL	1 mL
24-well (2 cm^2)	0.2 mL	0.5 mL	0.2 mL	0.5 mL
35-mm (10 cm^2)	1 mL	2 mL	1 mL	2 mL
60-mm (20 cm^2)	2 mL	4 mL	2 mL	4 mL
100-mm (60 cm^2)	6 mL	12 mL	6 mL	12 mL
T-25 (25 cm^2)	2.5 mL	4–5 mL	3 mL	4–5 mL
T-75 (75 cm^2)	7.5 mL	12–15 mL	8 mL	12–15 mL

[1] The optimal working concentration of CTS™ VTN-N is cell line dependent.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A27940
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
CTS™ Versene Solution	A4239101
CTS™ PSC Cryomedium	A4238801
CTS™ RevitaCell™ Supplement	A4238401
CTS™ PSC Cryopreservation Kit	A4239301
rhLaminin-521	A29249

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.