Essential 8™ Medium

Catalog Number A1517001

Pub. No. MAN0007569 Rev. 4.0

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™ Essential 8™ Medium (Cat. No. A1517001) is a serum-free, xeno-free medium that supports the culture of pluripotent stem cells (PSCs). Unlike most feeder-free media, the xeno-free Essential 8™ Medium does not require the presence of BSA (bovine serum albumin) or HSA (human serum albumin), minimizing batch variability and improving feeder-free culture conditions for pluripotent stem cells (PSCs).

Contents and storage

Contents[1]	Cat. No.	Amount	Storage	Shelf life[2]
Essential 8™ Medium, (Cat. No. A1517001)				
Essential 8™ Basal Medium	A1516901	500 mL	2°C to 8°C. Protect from light.	12 months
Essential 8™ Supplement (50X)[3]	A1517101	10 mL	-20°C to -5°C. Protect from light.	

[1] 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 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 Essential 8™ Medium

Culture type: Adherent

Recommended substrates: Vitronectin (Cat. No. A14700)

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.

Prepare complete Essential 8™ Medium (500 mL)

1. Thaw the frozen Essential 8™ Medium 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 Essential 8™ Basal Medium, then aseptically transfer 10 mL of Essential 8™ Supplement to the bottle of Essential 8™ Basal Medium.

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

5. Complete Essential 8™ Medium can be stored at 2°C to 8°C for up to 2 weeks. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch.

   Do not warm the medium at 37°C.

Guidelines to culture human PSCs in 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 Essential 8™ Medium

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

   See *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 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 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 1 mL of complete Essential 8™ Medium by gently pipetting the cells up and down a few times.

9. Slowly add the PSC suspension into a pre-warmed, VTN-N-coated 6-well plate containing 1 mL of Essential 8™ Medium per well, plating 1 vial of ~1 million viable thawed cells per well of a 6-well plate.

   Optional: To improve efficiency of cell survival 24 hours post-thaw, chemically defined, animal origin-free, RevitaCell™ Supplement (Cat No. A26445) may be added at 1X final concentration to the cell culture (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-thaw to minimize apoptosis and necrosis. Using this supplement for the recovery of PSCs requires a lower seeding density; therefore, seed 1 vial containing ~1 million viable cells across two wells of a 6-well plate (i.e., 2-fold lower cell seeding density than for recovery in Essential 8™ Medium alone).

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 Essential 8™ Medium.

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

Passage PSCs with Versene Solution

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

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

2. Aspirate the spent medium from the vessel containing PSCs and rinse each well twice with DPBS, no calcium, no magnesium.

3. Add the 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 Versene Solution and add pre-warmed complete Essential 8™ Medium to the vessel.

6. Remove the cells from the well(s) by gently squirting medium over the surface of the well a few times and pipetting the colonies up with a 5-mL glass pipette.

   Avoid creating bubbles.

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

   There may be obvious patches of cells that were not dislodged and left behind. Do not scrape the cells from the dish in an attempt to recover them. Two to three triturations should be sufficient. Do not pipet vigorously or the colonies will break apart.

   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 Essential 8™ Medium to the well(s), which quickly neutralizes the initial effect of the 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 Essential 8™ Medium to the 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 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, RevitaCell™ Supplement (Cat. No. A2644501) may be used at 1X final concentration (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-passage.

Recommended plating volumes

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

Culture vessel (approx. surface area)	1X Vitronectin solution[1]	DPBS	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	2–3 mL	4–5 mL
T-75 (75 cm^2)	7.5 mL	12–15 mL	5–8 mL	12–15 mL

[1] The optimal working concentration of VTN-N is cell line dependent. We recommend using a final coating concentration of 0.1–1.0 µg/cm^2 on the culture surface, depending on your cell line.

Related products

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

Item	Source
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
DPBS, no calcium, no magnesium	14190144
Versene Solution	15040066
RevitaCell™ Supplement	A2644501
PSC Cryopreservation Kit	A2644601

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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