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Cellular Dynamics international

Essential 8™ Medium (Prototype)

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Description

Essential 8™ Medium 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).

Kit Name/Components*	Catalog no./Part no.	Amount	Storage†
Essential 8™ Medium includes:	**A15045SA**	**1 kit**	
DMEM/F-12 (HAM) 1:1	A14625DJ	500 mL	Store at 2°C to 8°C. Protect from light.
Essential 8™ Supplement (50X)	A14626SA	10 mL	Store at –5°C to –20°C. Protect from light.

* Essential 8™ Medium is sold as a complete kit; its components are not available separately.

† Do not subject Essential 8™ Supplement to repeated freeze/thaw cycles. Store the supplement in a **non-frost-free** freezer at –5°C to –20°C.

Product Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses. Product is a prototype. Performance characteristics of this product have not been established by Life Technologies.

Handling

Thaw frozen Essential 8™ Supplement at 2°C to 8°C overnight before using it to prepare complete medium. **Do not thaw the frozen supplement at 37°C.**

Physical Conditions

Standard physical growth conditions for human pluripotent stem cells (PSCs) in complete Essential 8™ Medium are 37°C in a humidified atmosphere of 5% CO_2. Using standard aseptic conditions, cultures may be grown in complete Essential 8™ Medium on vitronectin (VTN-N)-coated, tissue culture-treated vessels. Ensure that proper gas exchange is achieved in culture vessels. Avoid overexposure of cultures to light.

Guidelines for Human PSCs Culture in Essential 8™ Medium

- Split cultures when the first of the following occurs:
  (a) PSC colonies are becoming too dense or too large;
  (b) PSC colonies are showing increased differentiation;
  (c) 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**.

Preparing Complete Essential 8™ Medium (500 mL)

1. Thaw the frozen Essential 8™ Supplement at 2°C to 8°C overnight. **Do not thaw the frozen supplement at 37°C.**
2. Mix the thawed supplement by gently inverting the vial a couple of times, remove 10 mL from the bottle of DMEM/F-12 (HAM) 1:1, and then aseptically transfer the entire contents of the Essential 8™ Supplement to the bottle of DMEM/F-12 (HAM) 1:1. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.
3. 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.**

Recovering Frozen PSCs in Complete Essential 8™ Medium

1. Pre-warm complete Essential 8™ Medium and VTN-N-coated 6-well plates to room temperature.
   Note: Refer to the Vitronectin (VTN-N) user guide for the coating procedure (available at **www.lifetechnologies.com**).
2. Remove the vial of PSCs from liquid nitrogen storage and transfer it on dry ice to the cell culture hood.
3. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently. When only an ice crystal remains, remove the vial from the water bath, spray the outside of it with 70% ethanol, and place it in the hood.
4. Transfer the thawed cells to a 15-mL conical tube and slowly add 10 mL of Essential 8™ Medium drop-wise to cells. This reduces osmotic shock to the cells. While adding the medium, gently move the tube back and forth to mix the PSCs. Rinse the vial with 1 mL of Essential 8™ Medium and add to the 15-mL tube with cells.
5. Centrifuge the cells at 200 × *g* for 5 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 2 mL of Essential 8™ Medium by gently pipetting the cells up and down a few times.
6. Slowly add the PSC suspension into pre-warmed, VTN-N-coated 6-well plate, plating 1 vial of thawed cells per well of 6-well plate.
7. Move the plate in several quick figure eight motions to disperse cells across the surface of the wells and place it gently into the 37°C, 5% CO_2 incubator.
8. 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.

Passaging PSCs Using EDTA

1. Prepare 0.5 mM EDTA by combining 50 µL of UltraPure™ 0.5 M EDTA, pH 8.0 with 50 mL of DPBS without Calcium and Magnesium. Filter sterilize the solution and store at room temperature.
2. Pre-warm complete Essential 8™ Medium and VTN-N-coated culture vessels to room temperature.
3. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel twice with DPBS without Calcium and Magnesium (refer to Table 1 for the recommended volume).
4. Add 0.5 mM EDTA in DPBS to the vessel containing PSCs (refer to Table 1). Swirl the vessel to coat the entire cell surface.
5. 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 in them when viewed under a microscope, they are ready to be removed from the vessel.

   Note: In larger vessels or with certain cell lines, this may take longer than 5 minutes.
6. Aspirate the EDTA solution, and add pre-warmed Essential 8™ Medium to the vessel (refer to Table 1).
7. Remove the cells from the well(s) by gently squirting medium and pipetting the colonies up. Avoid creating bubbles. 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.**

   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 EDTA. 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 Essential 8™ Medium to each well of a VTN-N-coated 6-well plate so that each well contains 2 mL of medium after the cell suspension has been added. Refer to Table 1 for the recommended volumes for other culture vessels.
9. Move the vessel in several quick figure eight motions to disperse the cells across the surface of the vessels. Place the vessel gently into the 37°C, 5% CO_2 incubator and incubate the cells overnight.
10. Feed the PSC cells beginning the second day after splitting. Replace the spent medium daily.

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

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

Culture vessel (approx. surface area)	Vitronectin solution*	DPBS	0.5 mM EDTA in DPBS	Complete medium
6-well (10 cm^2/well)	1 mL	2 mL	1 mL	2 mL
12-well (4 cm^2/well)	0.4 mL	1 mL	0.4 mL	1 mL
24-well (2 cm^2/well)	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

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

Related Products

Product	Cat. No.
Vitronectin, truncated human recombinant (VTN-N)	A15068SA
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
UltraPure™ 0.5 M EDTA, pH 8.0	15575

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

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