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by Thermo Fisher Scientific

Essential 8™ Flex Medium

Description

Essential 8™ Flex Medium is a serum-free, xeno-free medium that supports the culture and expansion of pluripotent stem cells (PSCs) without the need for daily feeding, allowing for weekend-free maintenance/expansion of PSCs. Just like the original Essential 8™ Medium formulation (Cat. no. A1517001), the Essential 8™ Flex Medium does not include BSA (bovine serum albumin) or HSA (human serum albumin) components, minimizing batch variability and maintaining long term health and pluripotency of PSCs.

Product*	Catalog no.	Amount	Storage	Shelf life†
Essential 8™ Flex Medium Kit contains:	**A28585-01**	**1 Kit**		
Essential 8™ Flex Basal Medium	A28583-01	500 mL	Store at 2°C to 8°C. Protect from light.	12 months
Essential 8™ Flex Supplement (50X)**	A28584-01	10 mL	Store at –20°C to –5°C. Protect from light.	12 months

* Essential 8™ Flex Medium is sold as a complete kit; individual components are not sold separately. ** Store the Essential 8™ Flex Supplement in a **non-frost-free** freezer at –5°C to –20°C. Do not refreeze the thawed supplement. † Shelf life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

Thaw the frozen Essential 8™ Flex Supplement at room temperature for ~1 hour to prepare complete medium. Supplement may also be thawed at 2°C to 8°C overnight; small amounts of precipitate may be observed, but this will not affect product performance. **Do not thaw the frozen supplement at 37°C.**

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture conditions

Media: Complete Essential 8™ Flex Medium

Culture type: Adherent

Recommended substrate: Vitronectin (VTN-N) Recombinant Human Protein, Truncated (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™ Flex Medium (500 mL)

1. Thaw the frozen Essential 8™ Flex 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, remove 10 mL from the bottle of Essential 8™ Flex Basal Medium, and then aseptically transfer the entire contents of the Essential 8™ Flex Supplement to the bottle of Essential 8™ Flex Basal Medium. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.
3. Complete Essential 8™ Flex 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.**

Human PSCs culture in Essential 8™ Flex 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 3 to 4 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.
- Cells are normally split twice weekly, with medium exchanges completed 18–36 hours after plating to remove any resulting cell debris. Additional feeds, including those during the weekends, are not required. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume (see Figure 1, page 2).
- Newly derived PSC lines may contain a fair amount of differentiated cells 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 early passages.
- **Do not scrape the cells from the culture vessel during passaging**.

Recover frozen PSCs in complete Essential 8™ Flex Medium

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

   Note: Refer to the **Vitronectin (VTN-N) user guide** and Table 1 (page 2) for the coating procedure.
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. 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 complete Essential 8™ Flex Medium drop-wise to the 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 complete Essential 8™ Flex 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 complete Essential 8™ Flex 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 ~1 million viable thawed cells per well.
7. *(Optional):* To improve cell survival, you can use RevitaCell™ Supplement (Cat. no. A26445) at 1X final concentration in 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™ Flex Medium alone).

8. Move the plate in several quick 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.
9. The next day, replace the spent medium with fresh complete Essential 8™ Flex Medium.
10. *(Optional):* Replace the medium daily thereafter until the cells are ~85% confluent.

    Note: Additional feeds, including those during the weekends, are not required. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume (see Figure 1).

Passage PSCs using Versene

1. Pre-warm complete Essential 8™ Flex Medium, VTN-N-coated 6-well culture plate, and the Versene solution to room temperature.
2. Aspirate the spent medium from each well containing PSCs and rinse each well twice with DPBS without calcium or magnesium (refer to Table 1 for the recommended volume).
3. Add the Versene solution to each well containing PSCs (refer to Table 1). Swirl the vessel to coat the entire cell surface.
4. Incubate the plate 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 wells.
5. Aspirate the Versene solution, and add pre-warmed complete Essential 8™ Flex Medium to each well (refer to Table 1).
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. Avoid creating bubbles. Collect the 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 plate in an attempt to recover them. Do not over-triturate the cell suspension.**

    Note: Depending upon the cell line, work with no more than 1 to 3 wells at a time, and work quickly to remove the cells after adding Essential 8™ Flex Medium to the well(s), which quickly neutralizes the initial effect of Versene. Some lines re-adhere 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.
7. Add an appropriate volume of pre-warmed complete Essential 8™ Flex 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.
8. Mix the cell suspensions from step 6 by gentle inversion a few times and transfer the appropriate volume of cell suspension into each well containing pre-warmed complete Essential 8™ Flex Medium.
9. Move the plate in several quick side-to-side motions to disperse the cells across the surface of the wells. Incubate the cells in the 37°C, 5% CO_2 incubator overnight.
10. Feed the PSCs the day after splitting. Additional feeds, including those during the weekends, are not required. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume.
11. *(Optional):* To improve cell survival, you can add RevitaCell™ Supplement (Cat. no. A26445) to 1X final concentration (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-passage.

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

Figure 1 Typical weekly PSC culture workflow using the Essential™ 8 Flex Medium.

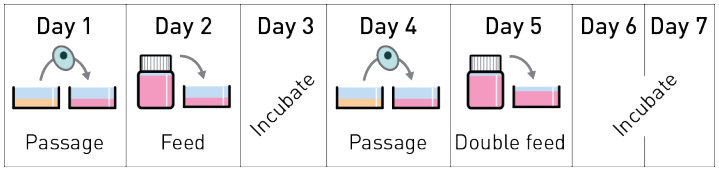

Table 1 Reagent volumes (in mL per well or per dish)

Culture vessel (approx. surface area)	Vitronectin solution*	DPBS	Versene solution	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 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

Product	Cat. no.
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
Versene Solution	15040
RevitaCell™ Supplement	A26445
PSC Cryopreservation Kit	A26446

Explanation of symbols and warnings

The symbols present on the product label are explained below:

Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
LOT	REF		STERILE A	Read SDS
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Limited product warranty

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